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THE UNIVERSITY OF ALBERTA

METABOLITES OF *TRICHODERMA LONGIBRACHIATUM*

BY

ROMANO ANDRADE VALVERDE

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND
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DOCTOR OF PHILOSOPHY

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(SIGNED): Romano Andrade

PERMANENT ADDRESS:

Av. 6, #1155,
San José, Costa Rica
Central America

DATE: August 28, 1989

THE UNIVERSITY OF ALBERTA
FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance , a thesis entitled METABOLITES OF *TRICHODERMA LONGIBRACHIATUM* submitted by ROMANO ANDRADE VALVERDE in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY.

Supervisor

R G Jordan

External Examiner

DATE: August 25, 1989

To Louise and Luis

ABSTRACT

Trichoderma longibrachiatum Rifai aggr.is a fungus which was reported to be antagonistic to the fungus *Mycena citricolor* (Berkeley & Curtis) Saccardo, the causative agent of the American leaf spot disease of coffee. We have investigated the metabolites of this fungus and have isolated the known compounds sorbicillin, bisvertinol, and bisvertinolone, and the new natural products trichodimerol, trichodermolide, sorbiquinol, bislongiquinolide, and 5-hydroxyvertinolide. The structures of the new compounds were determined by a combination of spectroscopic techniques, including ^{1}H and ^{13}C nmr, uv, CD, ORD, ir, hreims, and cims spectra, ^{1}H nmr solvent shifts, selective INEPT, natural abundance ^{13}C INADEQUATE, $[^{1}H]$-^{1}H and $[^{1}H]$-^{13}C nOe experiments, the formation of chemical derivatives, and biogenetic and symmetry considerations. The absolute stereochemistry of trichodimerol, trichodermolide, bisvertinol, and bisvertinolone was determined by CD spectroscopy.

Octahydrotrichodimerol has been prepared. Acetylation of octahydrotrichodimerol gave two unsymmetrical monoacetyl derivatives, a symmetrical diacetate, an unsymmetrical triacetate, and a symmetrical tetraacetate.

Octahydrotrichodermolide has been prepared and reduced with sodium borohydride to give two epimeric alcohols. Treatment of the epimeric alcohols with acid provided anhydro and aromatic derivatives.

Reduction of sorbiquinol afforded tetrahydro and hexahydro derivatives. The absolute stereochemistry has not been unambiguously determined but a tentative assignment is presented.

The relative stereochemistry of bisvertinol and bisvertinolone has been revised to an all cis stereochemistry and the structures of bislongiquinolide and 5-hydroxyvertinolide are tentatively assigned.

Finally, incorporation of [1-^{13}C]acetate into these compounds established their polyketide origin and allows us to present a biosynthetic hypothesis which accounts for most of the metabolites. Incorporation of [1,2-$^{13}C_2$]acetate into sorbicillin and trichodimerol provided support for the biosynthetic schemes.

The biosynthesis of trichodermolide remains unclear and requires further incorporation experiments. A biosynthetic hypothesis is presented.

ACKNOWLEDGEMENTS

I would like to thank:

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LIST OF ABBREVIATIONS

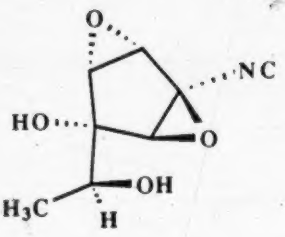
APT	attached proton test
ATCC	American Type Culture Collection
br.	broad
ca	calculated
CD	circular dichroism
CMI	Commonwealth Mycological Institute
cims	chemical ionization mass spectrum
COSY	*CO*rrelation *S*pectrocop*Y*
hreims	high resolution mass spectrum
INADEQUATE	*I*ncredible *N*atural *A*bundance *D*oubl*E* *QUA*ntum *T*ransfer *E*xperiment
INADSYM	symmetrizable INADEQUATE
INAPT	*I*nsensitive *N*uclei *A*ssignment by *P*olarization *T*ransfer
INEPT	*I*nsensitive *N*uclei *E*nhancement by *P*olarization *T*ransfer
ir	infrared
J	coupling constant
nmr	nuclear magnetic resonance
nOe	nuclear Overhauser effect
ORD	optical rotatory dispersion
PDA	potato dextrose agar
QM	Quartermaster Research and Development Center
sh	shoulder

tlc	thin layer chromatography
UAMH	University of Alberta Microfungus Collection and Herbarium
uv	ultraviolet

INTRODUCTION

Species of the genus *Trichoderma* Persoon are numerous and widespread, and their taxonomic classification difficult. This difficulty has led to the development of a *species aggregate* system of classification (1) which groups together several "species". Rifai has noted: "Since it is admitted that under the name *Trichoderma hamatum* (Bon.) Bain aggr., for example, there may be two or three or more different but morphologically very similar species, it follows that isolates considered to belong to this species aggregate may behave quite differently under different conditions." (2). It is not surprising, then, that there is no clear pattern among the secondary metabolites produced by *Trichoderma* strains.

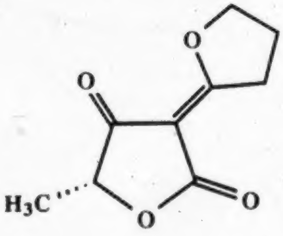
The genus *Trichoderma* produces a wide range of natural products. Among these are: isonitrile antibiotics (e.g. trichoviridin (**I**)), produced by strains of *T. hamatum*, *T. harzianum* Rifai aggr., *T. viride* Pers. ex S.F. Gray aggr., *T. koningii* Oudem. aggr., and *T. polysporum* (Link ex Pers.) Riffai aggr. (3, 4); antibiotic polypeptides (e.g. alamethicin (**II**)), produced by strains of *T. viride*, *T. polysporum* (also known as *Tolypocladium inflatum*, 5), and *T. harzianum* (6); carolic acid (**III**) (7), trichodermin (**IV**), a trichothecane (8), and gliotoxin (**V**) (9), produced by unidentified *Trichoderma* strains; alpha pyrones (e.g. **VI**), produced by *T. viride*, *T. koningii* and *T. harzianum* (10, 11, 12); terpenes (e.g. avocettin (**VII**) from *T. viride*) (13); and polyketides (e.g. **VIII**, **IX**, and **X** from *T. viride*, *T. pseudokoningii* Rifai aggr., and *T. koningii*) (12, 14, 15).



I

Ac Aib L-Pro Aib L-Ala Aib L-Al L-Gln Aib L-Val Aib
L-Phol-Gln-Glu Aib-Aib L-Val-Pro Aib L-Leu Gly

II



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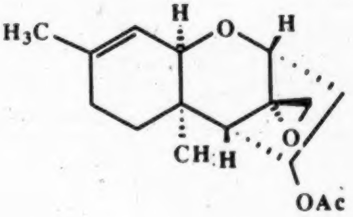

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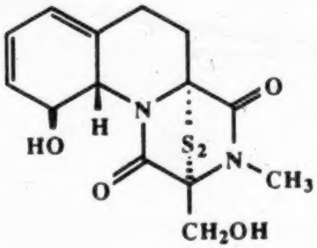

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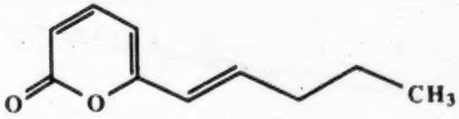


VI

In spite of the importance of the genus, there is no chemical work reported on species of *T. longibrachiatum*, or on other "yellow" *Trichoderma* strains. Rifai (1) has documented the existence of yellow pigments in *Trichoderma* strains of the aggregates species *T. aureoviride*, *T. longibrachiatum*, and *T. harzianum*. Furthermore, Rifai records that the type culture of *T. longibrachiatum* Rifai aggr. did not produce yellow pigments, but colonies of an isolate, referred to by him as *Dingley 9*, had a "lemon yellow reverse". He argues that the ability of certain strains of *T. viride* to change the color of the media on which they are grown indicates that little taxonomic significance need be given to the pigments produced by *Trichoderma*.

Trichoderma strains have been shown to be antagonistic to other fungi. Among these are plant pathogenic fungi of economic importance: *Rhizoctonia solani* Kuhn (damping-off and root rot of many crops), *Sclerotium rolfsii* Saccardo (stem blight of peanuts), *Pythium* sp. (damping-off of seedlings), *Verticillium fungicola* (Preuss) Hassebrauk (dry bubble cultivated in mushrooms), *Armillaria mellea* (Vahl: Fr.) Kummer (root disease in trees), *Chondrostereum purpureum* (Pers: Fr) Pouzar (silverleaf disease of plum), *Heterobasidon annosum* (Fries) Brefeld (root disease of trees), *Botrytis cinerea* Pers. ex Nocca & Balb. (grey mold rot), *Phomopsis viticola* Saccardo (dead arm disease of grape), *Pyricularia oryzae* Cavara (rice pathogen), *Mucor mucedo* Mich. ex St. Am. (fruit spoilage), and *Phytophthora infestans* (Mont.) de Bary (late blight of potato) (16, 17). For this reason, *Trichoderma* strains have been considered to have potential as biocontrol agents, and in some cases they appear to be effective as such.

Our interest in the chemistry of the metabolites produced by *T. longibrachiatum* was stimulated by its potential use as a biocontrol for the leaf spot disease of coffee (ojo de gallo), caused by the fungus *Mycena citricolor* (Berkeley & Curtis) Saccardo. As part of an international collaborative project between the University of Costa Rica and the University of Alberta, Professor Edgar Vargas of the University of Costa Rica pointed out to us that when grown in agar plate co-cultures, *T. longibrachiatum* caused lysis of the hyphae of *M. citricolor*. This observation prompted us to consider *T. longibrachiatum* as a potential biocontrol agent for *M. citricolor*.

Unfortunately the antagonistic activity sometimes observed with *T. longibrachiatum* against *M. citricolor* was not observed with the extracts obtained when *T. longibrachiatum* was grown in still or shake culture. However, the isolation of some colorful and apparently structurally unique compounds from these extracts prompted us to examine the compounds in more detail.

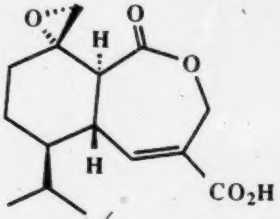
A brief description of some of the compounds which were encountered in the investigation of the metabolites of *T. longibrachiatum* is in order.

In 1948 Cram and Tishler (18) isolated sorbicillin (**XI**) from a sample of clinical penicillin and Cram (19) determined its structure by means of a series of chemical degradations. Sorbic acid (**XII**) was obtained from sorbicillin by reaction with alkaline hydrogen peroxide (Dakin reaction). This, plus its co-occurrence with penicillin, prompted the name. The structure of the aromatic portion of sorbicillin was established by comparison of the uv spectra of a product of alkaline degradation of sorbicillin and a synthetic sample of 2,4-dihydroxy-3,5-dimethylacetophenone (clavatol (**XIII**)), which had been isolated from *Aspergillus clavatus* Desm. by Hassall and Todd (20).

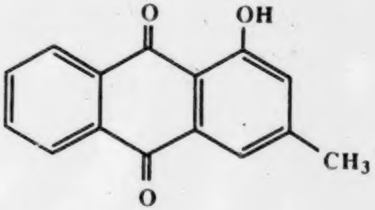
Sorbicillin was later isolated by Dreiding and co-workers (21a) from *Verticillium intertextum* Issac & Davis. Interestingly there is a high degree of morphological similarity among the genera *Trichoderma*, *Gliocladium* Corda, and *Verticillium* Nees ex Link (1) and species of these genera have been observed to produce similar compounds. For example, gliotoxin was isolated from a strain which was originally identified as *T. viride* but later reidentified as *Gliocladium virens* Miller, Giddens, and Foster (22). This supports the assumption, based on morphology, that there is a close taxonomic relationship between the genera *Gliocladium* and *Trichoderma*.

In addition to sorbicillin, Dreiding and co-workers isolated a number of interesting metabolites from *V. intertextum*: dihydrosorbicillin (**XIV**), vertinolide (**XV**), bisvertinol (**XVI**), dihydrobisvertinol (**XVII**), isodihydrobisvertinol (**XVIII**), bisvertinolone (**XIX**), and bisvertinoquinol (**XX**) (21). The structures of vertinolide and bisvertinoquinol were determined by X-ray analysis (21b, 21c), while the structure of sorbicillin was confirmed as **XI** by synthesis (20). The structures of bisvertinol, dihydrobisvertinol, isodihydrobisvertinol, and bisvertinolone were determined by a combination of chemical conversions and spectroscopic characterization. Mass spectra and uv spectra were important in establishing the constitution of the unsaturated chains in these compounds. The relative stereochemistry of the "bisvertinols" was proposed by Dreiding and co-workers based on biogenetic arguments. While the absolute stereochemistry of vertinolide was established by synthesis (23), the absolute stereochemistry of the bisvertinols and bisvertinoquinol was not established.

With this background we embarked on the isolation, structure determination and study of the biosynthesis of the metabolites of *T. longibrachiatum*.



VII



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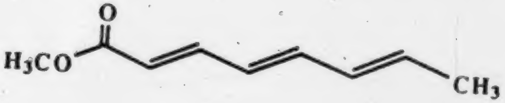

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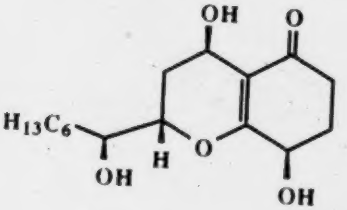
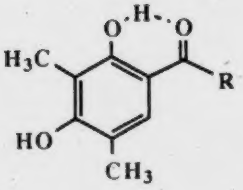
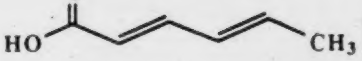


X



XI R = A
XVI R = B



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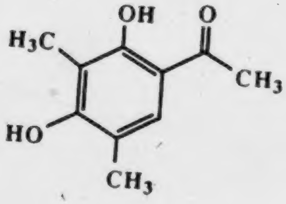

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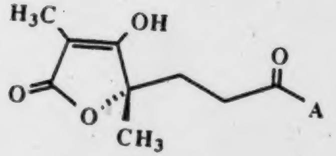

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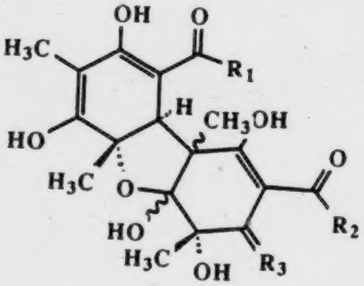
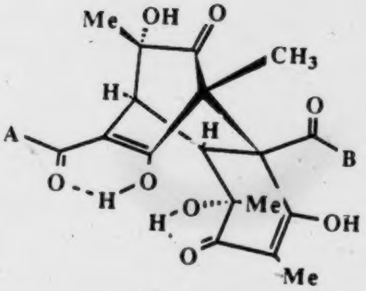
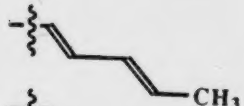


XVI R$_1$, R$_2$ = A, R$_3$ = H$_2$
XVII R$_1$ = A, R$_2$ = B, R$_3$ = H$_2$
XVIII R$_1$ = B, R$_2$ = A, R$_3$ = H$_2$
XIX R$_1$, R$_2$ = A, R$_3$ = O



XX

A =



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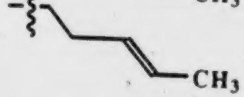

RESULTS AND DISCUSSION

In order to study the metabolites produced by *T. longibrachiatum* we decided to grow two strains in a semi-synthetic medium according to a recipe provided by the Costa Rican collaborators (see Experimental). The main carbon source of the medium were changed from glucose to lactose since there is a report (24) of increased glycosidase activity when lactose is the main carbon source.

After growing the fungus in still and shake cultures, harvesting the mycelium, and extracting the resulting broths and mycelia, we investigated several isolation procedures. In doing so we found that silica gel chromatography was not the purification method of choice. Mass balance of the fractions collected after silica gel chromatography indicated that there were significant amounts of material lost. As well, elution with very polar solvent mixtures and comparison of the ^{1}H nmr spectra of the fractions so obtained with the ^{1}H nmr spectra of the initial mixtures indicated that the metabolites had been denatured by the silica gel. An alternative approach was therefore necessary.

From these silica gel purifications, however, we were able to isolate a compound which was later identified as sorbicillin (*vide infra*). A search of the literature for metabolites related to sorbicillin indicated the success of other experimenters in isolating co-metabolites of sorbicillin by means of Sephadex LH-20 chromatography. Indeed Sephadex LH-20 chromatography proved to be a very useful tool in handling these silica-sensitive compounds. We developed an isolation procedure in which the metabolites of *T. longibrachiatum* could be separated after one Sephadex column with purification by a silica gel column for the compounds that can resist it or further Sephadex LH-20 chromatography.

The still cultures produce an array of different metabolites, while the shake cultures produce a more limited number of metabolites. Therefore, most of our work involved the isolation and structure determination of the still culture metabolites.

Sorbicillin

We isolated an orange compound from the mycelial extract of *T. longibrachiatum* (UAMH 5068). The structure of this orange compound was derived in the following way: It is an optically inactive compound with a molecular formula of $C_{14}H_{16}O_3$ as indicated by the high resolution mass spectrum (hreims) (m/z of 232.1096 calcd for $C_{14}H_{16}O_3$: 232.1100, 97%) and confirmed by chemical ionization mass spectrometry. Its infrared spectrum shows the presence of a strongly chelated carbonyl (1640 cm^{-1}) (25), an aromatic ring (1480, 1559, and 1620 cm^{-1}), and hydroxyl group (3360 cm^{-1}), while its uv spectrum shows a maximum at 318 (ε = 27000) nm which shifts to longer wavelengths upon addition of base (405 nm), thus indicating that the chromophore has acid properties. The ^1H nmr spectrum of sorbicillin shows a hydrogen signal assigned to a chelated phenol (δ 13.58 ppm) (26), signals suggestive of a dimethylphenol group {δ 2.16 (s, 3H), 2.24 (s, 3H), 5.26 (s, 1H), and 7.48 (s, 1H) ppm} (26, 27), and signals of a six carbon unsaturated chain. The olefinic signals show a coupling pattern consistent with a sorbyl* side chain {δ 6.32 (dq, 6, 15 Hz, 1H), 6.38 (dd, 10, 15 Hz, 1H), 6.98 ,(d, 15 Hz, 1H), and 7.48 (dd, 10, 15 Hz, 1H) ppm}. The stereochemistry of the unsaturated chain is assigned as E,E on the basis of the size of the coupling constants (15 Hz) (28) between the signals at δ 6.98 and 7.48 ppm and δ 6.38 and 6.32 ppm. These couplings were verified by decoupling experiments.

The chelated phenol requires the presence of two oxygens and the phenol of one. Since there are only three oxygens in the orange compound, the carbonyl observed in the ir spectrum must be part of the sorbyl side chain and hydrogen bonded to a phenol.

The ^{13}C nmr spectrum shows 14 carbon** signals. The chemical shifts together with the multiplicities of the signals support the presence of a sorbyl side chain and the aromatic group. Of specially interest is the presence of a methyl carbon signal at δ 7.0 ppm. The chemical shift is at rather high field, suggesting it is sterically crowded (30).

The arrangement of the substituents in the aromatic ring of the orange compound was investigated by the use of nOe*** difference experiments. The difference technique was used throughout, rather than the simple integration of the nmr signal since: "...by the difference procedure, enhancements of only 0.5%, as opposed to 10%, can be reliably observed". (32). Since nuclear Overhauser effects depend on the dipolar coupling of the nuclei involved and the tumbling in solution of the compound, and the coupling varies in most cases as the inverse of the separating radius to the sixth power, only nuclei in close proximity give rise to observable nOe enhancements. If the tumbling is slowed down by the viscosity of the solution or a high molecular weight of the compound, nOe enhancements can be very small, zero or even display a "negative enhancement" (33).

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* E,E-hexa-2,4-dienoyl.

** The ^{13}C multiplicities were verified by use of the spin-echo technique initially developed in this department and known as APT (29).

*** nOe: "is the change in the integrated intensity of the nmr absorption of a nuclear spin as a result of the concurrent saturation of another nmr resonance." (31)

The results of the nOe experiments on the orange compound were as follows: Irradiation of the aromatic methine (δ 7.48 ppm) in benzene-d_6 gave a large enhancement (31%) of one of the sorbyl hydrogens (δ 6.98 ppm), while irradiation of the methyl at δ 2.24 ppm gave enhancement of the aromatic methine and the phenol (δ 5.26 ppm). The other aromatic methyl group (δ 2.16 ppm) only gave enhancement of the phenolic hydroxyl hydrogen.

These results suggest that the orange compound has the structure **1**, previously known from natural sources as sorbicillin.

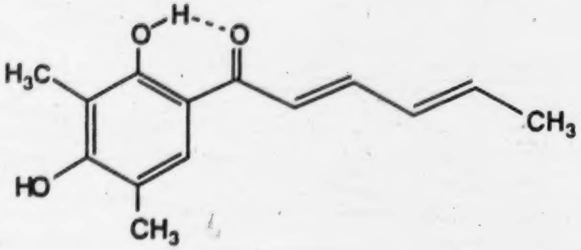
Further evidence for the assigned structure of the orange compound was obtained from its mass spectrum. In addition to the molecular ion, the hreims shows the loss of methyl (m/z 217, 100%), the sorbyl side chain plus one hydrogen (m/z 136, 72%), and $C_9H_9O_3$ (m/z 165, 68%). The ion at m/z 165 could be the stabilized acylium ion **2** which gives further support to the structure.

The spectroscopic data of the orange compound (uv, ^1H nmr, ^{13}C nmr, ir, and hreims) are in agreement with that reported for sorbicillin (21d).

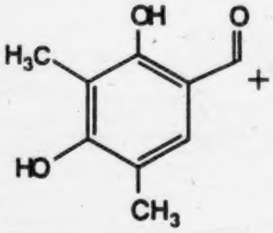
Finally, hydrogenation of the orange compound over palladium on carbon yielded tetrahydrosorbicillin (**3**) similar in all respects (uv, ir, ^1H

nmr, hreims, and ^{13}C nmr spectra) to that obtained by Dreiding and co-workers (21d). Thus the orange compound is sorbicillin (**1**).

Sorbicillin (**1**) was first isolated by Cram & Tishler in 1948 (18) from clinical penicillin* where it was present as an impurity. Cram used chemical methods (19) to elucidate its structure. Since then it has been synthesized by McOmie and Tute (35) and isolated from cultures of *Verticillium intertextum* by Dreiding and co-workers (21a, 21d).



1



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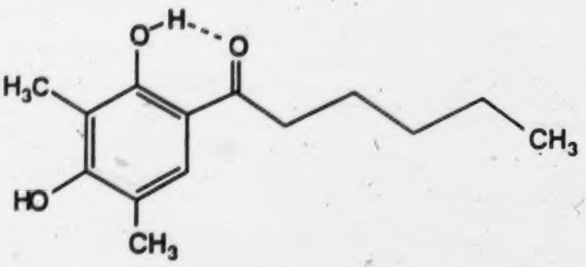

3

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* From a fungus initially known as *Penicillium notatum* but later as *Penicillium chrysogenum* Thom (34).

Trichodimerol

We have also isolated from the mycelial extract an unusual polyketide which we have named trichodimerol.

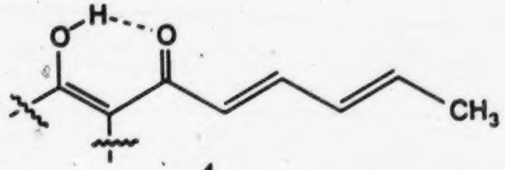
Trichodimerol is a ferric chloride positive, pale yellow, optically active compound ($[\alpha]_D$ -376°, *c* 0.26, MeOH), with a melting point of 156-157° C (benzene). Its uv spectrum shows absorptions at 362 (log $\varepsilon = 4.48$), 307 (4.20), 295 (4.17), and 240 (4.00) nm. The uv remains unchanged in the presence of acid. The maxima shifts to longer wavelengths in the presence of base (378 nm) indicating that the chromophore has acid characteristics. The ir spectrum shows the presence of a hydroxyl group (3420 cm^{-1}) and a strongly chelated carbonyl group (1613 cm^{-1}).

The molecular formula, $C_{28}H_{32}O_8$, was obtained from the hreims and the molecular ion was confirmed by fast atom bombardment mass spectrometry (fabms) (497, M+1, 35%). The mass spectrum also shows fragment ions at 1/2 M^+ ion at m/z 248 (22%) and an ion at m/z 95 which may be attributed to a sorbyl chain (C_6H_7O, 100%).

The ^1H nmr spectrum of trichodimerol (Table 1) displays methyl singlets (δ 1.43 and 1.46 ppm), a methine singlet (δ 3.00 ppm), a hydroxyl hydrogen (δ 3.20 ppm), a chelated enol (enolized β-diketone) hydrogen (δ 16.33 ppm), and the signals characteristic of a sorbyl chain {δ 1.89 (d, 6.5 Hz, 3H), 6.11-6.34 (m, 3H), 7.32 (dd, 10, 15 Hz, 1H) ppm}. The signal for the enolized β-diketone and the hydroxyl hydrogens disappear upon addition of D_2O. The stereochemistry of the unsaturated chain was assigned as E,E on the basis of the magnitude of the coupling constants measured in benzene solution (see Experimental): 15 Hz between signals at δ 5.55 and 5.88 ppm and δ 6.25 and 7.38 ppm. These couplings were verified by decoupling experiments.

The ^{13}C nmr spectrum (Table 2) shows fourteen signals, indicating an element of symmetry in trichodimerol. The signals correspond to the carbons of the sorbyl chain (δ 197.9, 143.6, 140.3, 131.0, 118.3, and 18.9 ppm), the methyls (δ 18.7 and 21.30 ppm), the methine (δ 57.6 ppm), and a series of fully substituted carbons (δ 58.9, 78.9, 102.8, 104.1, and 176.0 ppm). The carbon signal at δ 102.8 ppm can be assigned to the electron rich carbon of the enolized β-diketone system observed in the ^{1}H nmr, the signal at δ 58.9 to a non-oxygenated aliphatic carbon, the signal at δ 78.9 to a monooxygenated aliphatic carbon, the signal at δ 104.1 ppm to a dioxygenated carbon, and finally, the signal at δ 176.0 ppm to an oxygen bearing carbon of the enolized β-diketone system.

Since there are only two oxygen bearing sp^2 carbons (δ 197.9 and 176.0 ppm), we may conclude that the carbonyl of the sorbyl chain is part of the enolized β-diketone system. These data indicate the presence of fragment **4**, an enol-sorbyl system, in trichodimerol.

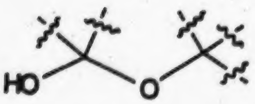


4

The molecular formula of trichodimerol indicates it has thirteen unsaturations. Since trichodimerol is dimeric, eight unsaturations may be attributed to the two enol-sorbyl systems (**4**), leaving five unassigned unsaturations, indicating that trichodimerol is pentacyclic.

There are eight oxygen atoms present in trichodimerol, four of them form part of the enol-sorbyl systems. This leaves four oxygens which are not accounted for. The ^{13}C nmr spectrum of trichodimerol has only two other signals attributed to oxygen bearing carbons (the monooxygenated and dioxygenated carbons at δ 78.9 and 104.1 ppm). Thus the remaining four oxygens must be present as two hemiacetals (fragment **5**).

In order to obtain further information on the relative position of the groups observed in the ^{1}H nmr spectrum, a series of nuclear Overhauser effect (nOe) difference experiments was carried out (Table 3). There is a large nOe enhancement between the δ 3.00 ppm methine and the sorbyl hydrogen at δ 6.12 ppm. which suggests that fragment **6** is present in trichodimerol. The methine at δ 3.00 ppm also gives nOe enhancements with the methyl groups at δ 1.43 and 1.46 ppm.



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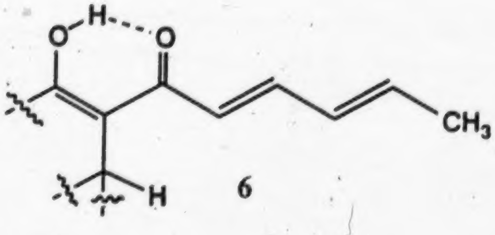


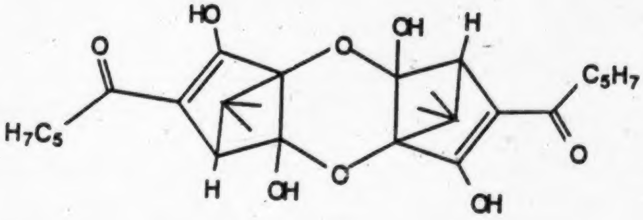
6

When pyridine is used as a solvent in nmr studies, it has been shown that the pyridine hydrogen bonds with hydroxyl groups, thereby affecting the chemical shift of hydrogens in the vicinity of the hydroxyl. The effect arising from this complexation is a downfield shift of the nearby hydrogens as a result of the anisotropic field of the pyridine (36).

When pyridine is used as the solvent for the ^{1}H nmr spectrum of trichodimerol, the methyls, the methine, and the hydroxyl group (hydrogen bonding), are significantly shifted (by 0.44, 0.57, 0.64, and 1.87 ppm respectively). These shifts are considerably larger than those observed when benzene is used as solvent, suggesting that the methyl groups are vicinal, 1,3-, or in some other way close to the hemiacetal hydroxyl (36).

At this stage it is possible to consider several structures for trichodimerol: **7**, **8**, **9**, **10**, and **11**.

Since there are only 14 signals in the ^{13}C nmr and the mass spectrum shows that trichodimerol is a 28 carbon compound, there must be an element of symmetry present in the structure. This element of symmetry must be a proper axis of symmetry since an improper axis of symmetry (a plane of symmetry and an inversion center are special cases of an improper axis of symmetry) would render the compound optically inactive (37) and this is not the case (vide supra).



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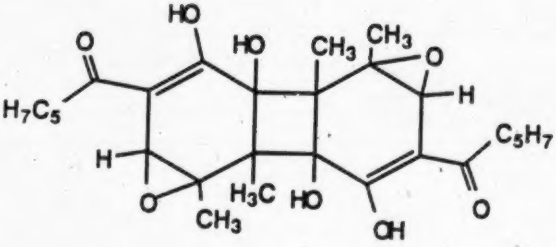

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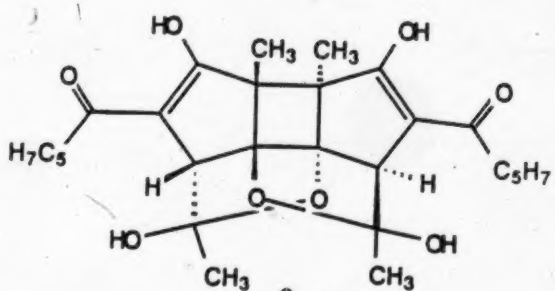

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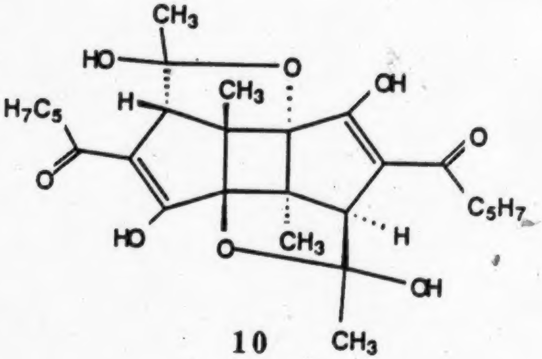

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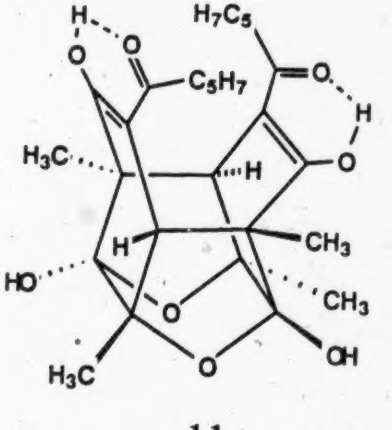
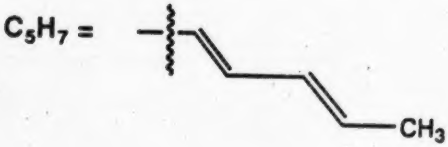


11



Among the various possible structures, **11** is chosen as the most likely structure of trichodimerol. This conclusion is based on the following: 1) The possible biogenesis of trichodimerol from sorbicillin: Trichodimerol may be a dimeric adduct of sorbicillin on the basis of the molecular formula and the functional groups present. Trichodimerol and sorbicillin both have "two" methyls as singlets, "a" sorbyl side chain, "a" methine, and are highly unsaturated compounds. Structure **11** is a dimer of two oxidized sorbicillin units. 2) Isomerism: Compounds like **9** and **10** should give rise to several stereoisomers differing at the hemiacetal centers, and these are not observed. 3) Chemical reactivity: Compound **8** should be extremely labile since the epoxide should open easily due to its proximity to the "enol". Similarly the monomer of **7** should be readily obtained and it is not. 4) Symmetry considerations: structure **10** is discarded since it has a center of inversion. Therefore, trichodimerol is assigned structure **11**.

Trichodimerol is characterized by its instability towards acids, which induce it to polymerize*. When left for some time in the absence or presence of solvent it decomposes to give an insoluble brittle solid. It was thus desirable to form a stable derivative of trichodimerol.

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* Sorbic acid and related ketonic compounds are known to polymerize (38).

Octahydrotrichodimerol (12).

As expected, hydrogenation over palladium on carbon yielded an octahydro derivative (**12**). The molecular formula, $C_{28}H_{40}O_8$, was obtained from the hreims. The uv spectrum shows a change in the chromophore with maxima now at 340 (390) and 294 (14000) nm (Table 4), consistent with a enolized β-diketone chromophore (39). The magnitude of the extinction coefficient indicates the system is fully enolized (39).

The ^1H nmr spectrum (Table 1) shows the signals of a five carbon saturated chain: a triplet for three hydrogens at δ 0.92 ppm, a multiplet for four hydrogens at δ 1.25 ppm, a multiplet for two hydrogens at δ 1.55 ppm, and a multiplet for the two diastereotopic C-8 hydrogens at δ 2.40-2.55 ppm (40); a methine singlet at δ 3.00 ppm; a hydroxyl hydrogen at δ 3.28 ppm; a six hydrogen singlet at δ 1.46 ppm (which may be resolved into two singlets when either pyridine or benzene are used as solvent); an enolized β-diketone hydrogen at δ 16.66 ppm; and no olefinic absorptions. The product retained its symmetry as shown by its ^{13}C nmr spectrum (Table 2, only 14 signals).

Octahydrotrichodimerol (**12**) is much more stable than trichodimerol (**11**) towards acid and base. As in trichodimerol the methyl groups and the methine hydrogen of octahydrotrichodimerol (**12**) display large shifts in the ^1H nmr spectrum when pyridine is added. The methyl groups and the methine gave mutual nOe* enhancements which are summarized in Table 3, together with the other observed enhancements.

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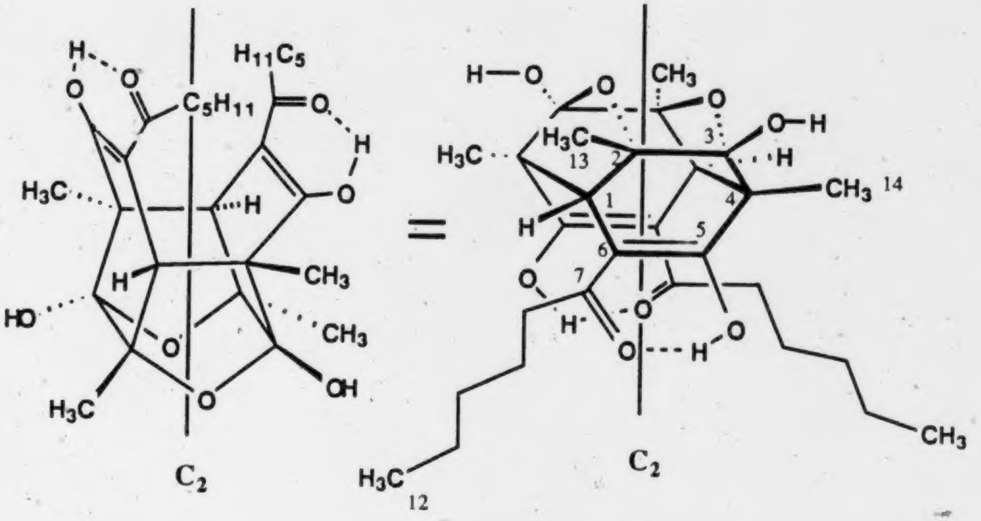
* Verified in benzene-d_6 where the two methyls give separate signals.


H₁₁C₅
C₅H₁₁
H₃C
HO
H₃C
CH₃
CH₃
OH
C₂
=
H—O
CH₃
O—H
H₃C
H₃C
13
2
3
1
4
5
6
7
CH₃ 14
H₃C 12
CH₃
C₂

12

Because of its enhanced stability, compound **12** was used for additional spectroscopic studies and for the preparation of several acetate derivatives.

Further evidence for the structure of trichodimerol was obtained from a ^{13}C-[^{1}H] nOe enhancement experiment and a natural abundance ^{13}C INADEQUATE experiment on octahydrotrichodimerol.

The location of the methine observed in the ^{1}H nmr spectrum relative to the non-hydrogen bearing carbons was investigated by the use of a ^{13}C-[^{1}H] nOe difference experiment. Selective irradiation of H-1 (δ 3.00 ppm, methine hydrogen) of octahydrotrichodimerol in benzene*, gave enhancements to the electron rich carbon of the enolized β-diketone system at δ 103.3 ppm (C-6), the monooxygenated carbon at δ 79.7 pm (C-2), the non-oxygenated quaternary carbon at δ 57.5 ppm (C-4), the side chain carbon at δ 31.7 ppm (C-8), and a negative enhancement to the dioxygenated carbon at δ 104.8 ppm (C-3).

The nOe to the carbon at δ 104.8 ppm is interesting. It must arise from an homonuclear nOe between H-1 and the two adjacent methyls (Me-13 and Me-14) followed by an heteronuclear nOe onto the carbons adjacent to the methyls. As well, the homonuclear nOe between the methine and the methyls is observed in the ^{1}H-[^{1}H] nOe experiments. Therefore, this negative nOe is an indirect one. Indirect nOe enhancements are known to occur for three spin systems (41, 42), including heteronuclear cases (43a).

There are very few reports of the use of the selective hydrogen-carbon nOe technique in structure elucidation (43), although the heteronuclear nOe has been known for some time**. This "new" technique is based on the selective irradiation of a hydrogen resonance with a "soft" decoupler power. This allows for the build-up of nOe enhancements to the carbons near that hydrogen, followed by a "read" pulse on carbon. The acquisition is done with "broad band" decoupling in order to give a hydrogen decoupled carbon spectrum. Because of this decoupling, the time between pulses must be long (45), usually more than ten seconds, in order to allow any generalized nuclear Overhauser effect from the strong decoupling to abate. During the waiting period the desired soft irradiation is retained in order to maintain the desired nOe. The waiting period reduces the usefulness of the technique for structure elucidation, since fewer acquisitions can be obtained, and thus large amounts of sample are required. Carbons one bond away from the irradiated hydrogen are not enhanced since the corresponding hydrogen signal is split into a doublet by the large one bond hydrogen-carbon coupling: the selective irradiation is too weak to include these "satellites".

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* The chemical shifts referred to are in benzene-d_6.

** As early as 1962 the enhancement of the ^{13}C signals by simultaneous hydrogen decoupling was noted (44).

Due to the complex nature of trichodimerol, we sought a further proof of the structure. The abundant sample of octahydrotrichodimerol justified a natural abundance ^{13}C two dimensional INADEQUATE experiment (46). This experiment can potentially establish the carbon-carbon connectivities and hence define the structure of trichodimerol. In an INADEQUATE experiment coupled carbons with coupling constants similar to the optimized coupling give pairs of signals, therefore, the carbon-carbon connectivity can be established. This experiment is known to require long acquisition times and large samples. These difficulties have precluded the widespread use of the INADEQUATE sequence in structure elucidation (47).

The carbons of interest in trichodimerol are quaternary. This requires the use of a long relaxation delay and precludes signal enhancement by nOe. Nevertheless an INADSYM (48) spectrum was obtained and is shown in Figure 1. The connectivities observed in the symmetrized spectrum* are between the carbon at δ 57.5 ppm (s) and the carbon at δ 104.8 ppm; between the carbon at δ 58.0 ppm (d) and the carbons at δ 103.3 and 79.7 ppm, and the carbon at δ 79.7 ppm and the carbon at δ 104.8 ppm. These connectivities prove that the methine carbon is bonded to the electron rich carbon of the enolized β-diketone system (δ 103.3 ppm) and the monooxygenated carbon (δ 79.7 ppm), and the quaternary carbon (δ 57.5 ppm) is bonded to the hemiacetal carbon. The INADSYM connectivities confirm that the structure of trichodimerol is as shown in **11**.

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* In benzene.

If an alternative assignment of the ^{13}C spectrum is made where the carbon at δ 104.8 ppm corresponds to the electron rich carbon of the enolized β-diketone system and the carbon at δ 103.3 ppm to the hemiacetal carbon, a conflicting situation arises which necessitates that the enolized β-diketone system be bonded to the monooxygenated and the quaternary carbons. Such a structural fragment is inconsistent with the other observations and therefore is discarded.

Some of the connectivities observed in the natural ^{13}C abundance INADEQUATE spectrum of octahydrotrichodimerol are confirmed by an INADEQUATE experiment of trichodimerol obtained from a [1,2-$^{13}C_2$] acetate feed culture growth (vide infra).

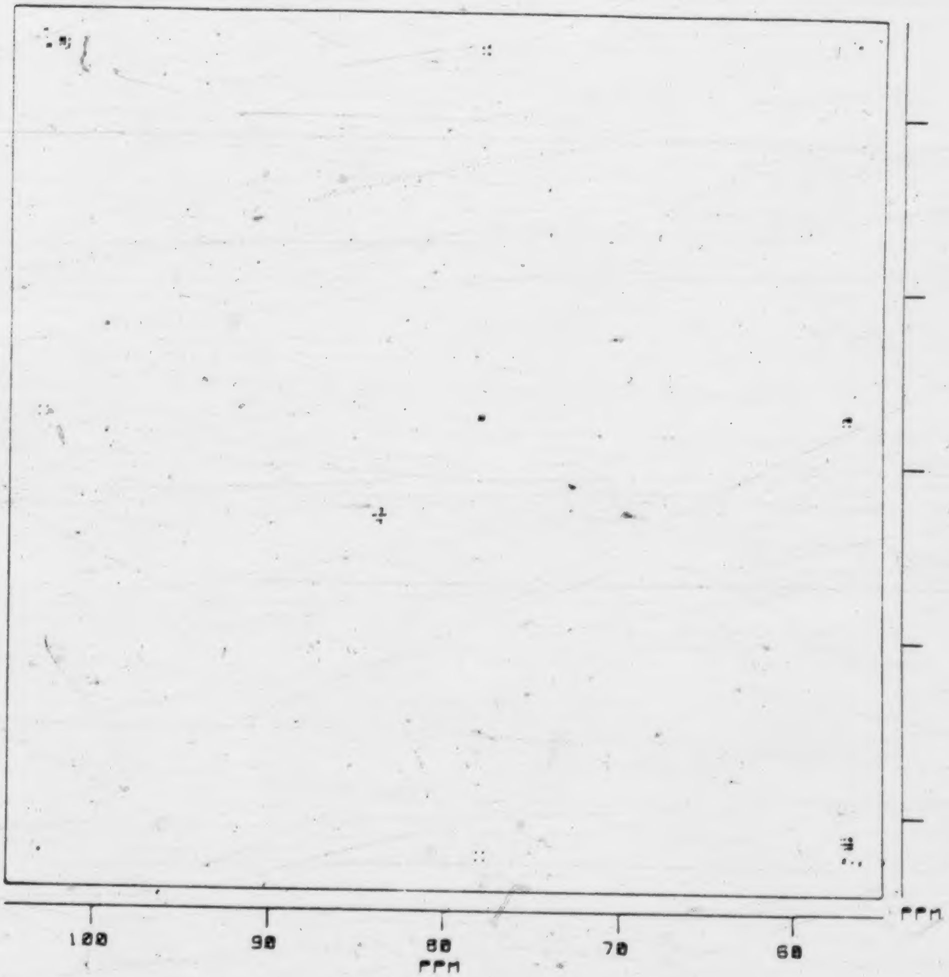


Figure 1. INADSYM spectrum of octahydrotrichodimerol (**12**) (C_6D_6, 90.56 MHz).

Acetylation of octahydrotrichodimerol (12).

Acetylation experiments with octahydrotrichodimerol were carried-out under several reaction conditions. Treatment of compound **12** with acetic anhydride in refluxing toluene or with acetic anhydride under reflux produced very complex product mixtures with at least 12 components as judged by tlc. When acetic anhydride, pyridine, and dimethylaminopyridine in dichloromethane were used for acetylation of **12**, five major acetylated products were isolated from the complex mixture formed.

Monoacetate 13.

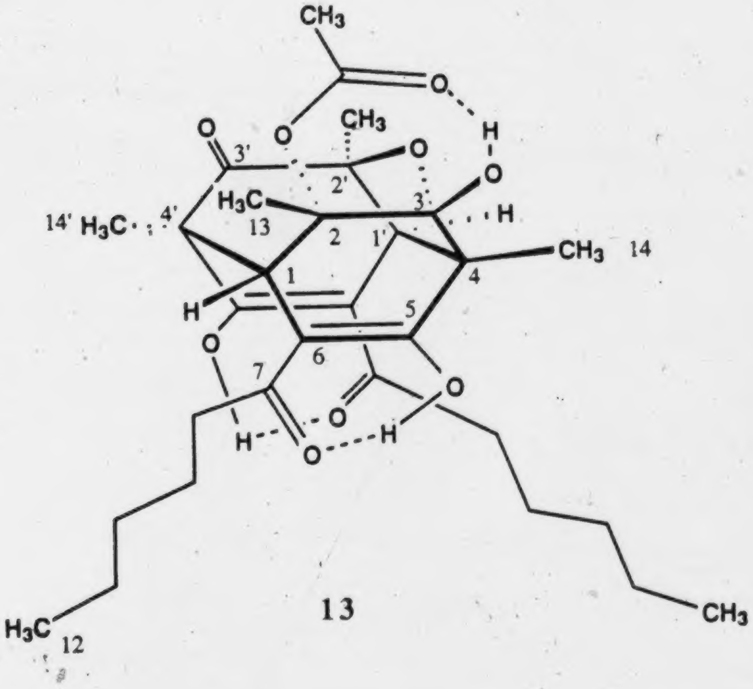
The molecular formula, $C_{30}H_{42}O_9$, obtained from the high resolution mass spectrum, as well as the ^{1}H nmr spectrum (δ 2.03 ppm, 3H) indicate that compound **13**, isolated from the acetylation reaction of octahydrotrichodimerol, is a monoacetate. The molecular ion was confirmed by cims. The ir spectrum of **13** shows the presence of a hydroxyl group (3400 cm^{-1}), a strongly chelated carbonyl group (1605 cm^{-1}), and an acetoxyl carbonyl group (1728 cm^{-1}, low frequency for an ester absorption). The uv spectrum of monoacetate **13** (Table 4) is similar to that observed for octahydrotrichodimerol (**12**), indicating that the enolized β-diketone observed in **12** is unchanged in compound **13**.

The ^{1}H nmr spectrum of monoacetate **13** (Table 1) displays methyl groups (δ 1.44, 1.469, 1.474, and 1.51 ppm), enolized β-diketone hydrogens (δ 15.94 and 16.23 ppm), a hydroxyl hydrogen (δ 6.12 ppm), and methine hydrogens (δ 2.98 and 3.19 ppm), all as sharp singlets. These and methine hydrogens (δ 2.98 and 3.19 ppm), all as sharp singlets. These indicate that compound **13** is no longer symmetrical, unlike its parent compound.

The ^{13}C nmr spectrum of **13** displays 29 signals, corresponding to 30 carbons. Five carbonyl-like absorptions are observed in the ^{13}C nmr spectrum (Table 2). It is apparent, however, from the integration of the signals that the signal at δ 188.4 ppm corresponds to two carbons. One of the carbonyls can be assigned to the newly introduced acetyl group (δ 173.3 ppm), another four arise from the two enolized β-diketone systems (δ 188.4 x 2, 195.1, and 196.5 ppm), and the sixth may be assigned to a ketone (δ 202.2 ppm). The presence of only three signals in the region δ 100-110 ppm indicates that compound **13** has a single hemiacetal functional group.

In order to establish the structure of compound **13** a series of nOe difference experiments was performed (Table 3). Among these a large nOe enhancement (12%) was observed between the methyl hydrogens at δ 1.44 ppm and the hydroxyl hydrogen at δ 6.12 ppm. This nOe is larger than would be expected for a freely rotating hydroxyl group. Also, the chemical shift of the hydroxyl hydrogen occurs at unusually low field, suggesting it is hydrogen bonded. The low energy absorption of the acetate carbonyl observed in the ir spectrum of **13** is consistent with this internal hydrogen bonding.

These observations, together with the isolation of another monoacetate (**14**) (described below), led us to conclude that under the reaction conditions used for acetylation of octahydrotrichodimerol (**12**), one of the hemiacetals of octahydrotrichodimerol opens to the hydroxy-ketone. The tertiary alcohol is then acetylated to produce **13**. The acetyl carbonyl is conveniently located to allow hydrogen bonding with the hydroxyl group.



13

The ^{13}C nmr chemical shifts of monoacetate **13** were compared with those of octahydrotrichodimerol (Table 2). A large downfield shift for C-2 from δ 78.8 to 89.0 ppm (10.2 ppm) is observed due to the acetylation of the tertiary alcohol (49). Other shifts observed are a downfield shift for C-4' from δ 58.0 to 63.8 ppm (5.8 ppm), and an upfield shift of C-1 and C-1' from δ 57.3 ppm to δ 53.3 and 56.8 ppm (0.5 and 4 ppm respectively) when **13** is compared with **12**. These shifts, α downfield and β upfield, are as expected for the change of a hemiacetal (in **12**) into a ketone (in **13**) (50).

Inspection of a molecular model of monoacetyloctahydrotrichodimerol (**13**) reveals that in the presence of hydrogen bonding, the hydroxyl and one of the methyls are proximate in space and this explains the large nOe enhancement mentioned above.

Furthermore, the nOe experiments (Table 3) allow the assignment of the methines and all the methyl hydrogen signals present in the ^{1}H nmr spectrum of monoacetate **13**. A diagram of the nOe enhancements is shown in Figure 2. From this network, beginning with the large nOe enhancement mentioned above, it is possible to proceed around the molecule and assign the ^{1}H nmr signals. On inspection of a molecular model of **13**, for example, the only methyl hydrogens that can give rise to the large nOe enhancement to the hydroxyl hydrogen is Me-13. Therefore, this is the methyl at δ 1.44 ppm. Methyl-13 shows an nOe enhancement with the δ 2.98 ppm methine hydrogen, thus this signal is H-1. The H-1 methine shows an nOe enhancement with the methyl hydrogens at δ 1.51 ppm, therefore, this signal is the Me-14'. A similar argument is used to assign the resonance of the other methyl hydrogens (Table 1).

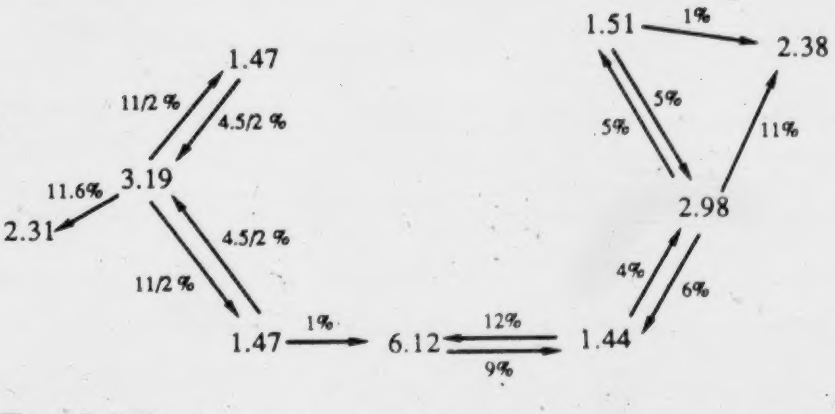


Figure 2. NOe enhancements observed in monoacetyloctahydrotrichodimerol (**13**) ($CDCl_3$, 360 MHz).

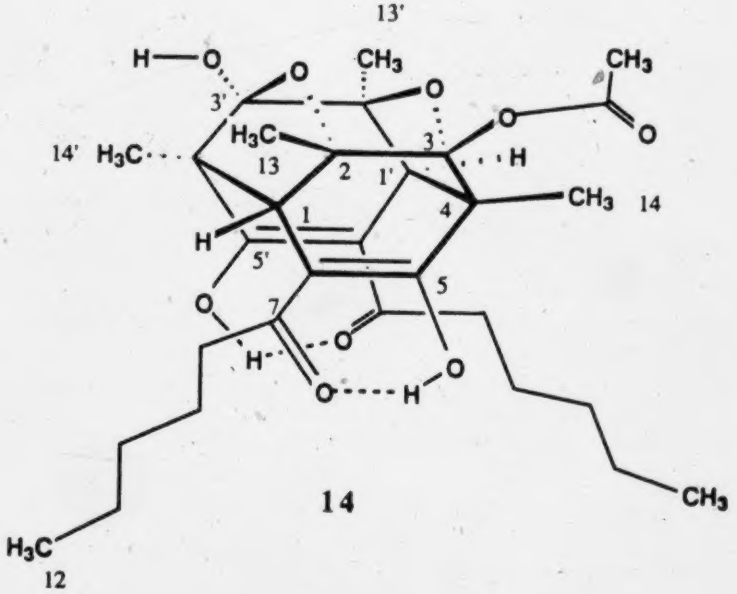
Monoacetate 14.

A second product obtained from the acetylation of octahydrotrichodimerol is also a monoacetate, **14**, as indicated by its molecular formula, $C_{30}H_{42}O_9$ (obtained from the hreims). In the ir spectrum a strongly chelated carbonyl (1600 cm^{-1}), hydroxyl (3440 cm^{-1}), and acetoxyl carbonyl (1752 cm^{-1}) absorptions are seen. The uv spectrum of **14** (Table 4) is similar to the uv spectrum of octahydrotrichodimerol (**12**), indicating that the two enolized β-diketone systems of **12** remain in compound **14**.

The ^1H nmr spectrum of **14** (Table 1) displays signals for hydrogens of methyls (δ 1.36, 1.41, 1.43, and 1.48 ppm), methines (δ 2.96 and 3.00 ppm), acetyl methyl (δ 2.11 ppm), enolized β-diketone hydrogens (δ 16.40 and 16.69 ppm), and a hydroxyl hydrogen (δ 2.97 ppm). These signals indicate that compound **14** is not a symmetrical molecule.

Twenty nine carbons are observed in the ^{13}C nmr spectrum of **14** (Table 2). There are five carbonyl-like carbon absorptions. One is attributed to the newly introduced acetyl carbonyl (δ 169.0 ppm) and four to the enolized β-diketone systems (δ 187.3, 191.8, 193.5, and 196.5 ppm). Furthermore, there are four carbon signals in the region 100-110 ppm suggesting that compound **14** has two hemiacetals.

Comparison of the ^{13}C nmr spectrum of **14** with that of **12** reveals that in **14** most ^{13}C nmr signals have shifted. Most notably an hemiacetal carbon shifts from δ 103.9 to 109.5 ppm (5.6 ppm). This shift is explained if the hemiacetal hydroxyl of **14** has been acetylated. It is expected that the increased electronegativity of the acetyl group would shift the hemiacetal carbon of **14** downfield (51) with respect to **12**. These observations indicate that compound **14** is an hemiacetal acetate of octahydrotrichodimerol.


13'
H—O
O
CH₃
CH₃
O
3'
O
H₃C
3
O
14' H₃C
13
2
1'
H
CH₃ 14
1
4
H
5'
5
O
7
O
H
O
O
H
H₃C
12
CH₃

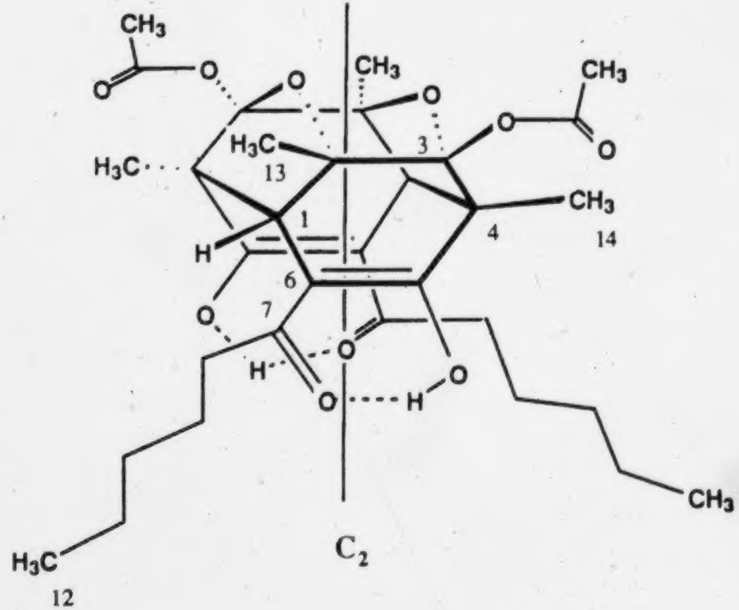
14

The isolation of two different monoacetates of octahydrotrichodimerol, one derived from an hemiacetal and another from an alcohol, is interesting and provides further evidence for the presence of an hemiacetal in trichodimerol.

Diacetate 15.

A third product obtained from the acetylation of octahydrotrichodimerol (**12**) is the diacetate **15**, $C_{32}H_{44}O_{10}$ (hreims, the molecular ion confirmed by cims). The ir spectrum shows an acetoxyl carbonyl (1753 cm^{-1}), a strongly chelated carbonyl (1599 cm^{-1}), but no O-H stretching absorptions above 3000 cm^{-1}. The uv spectrum of diacetate **15** (Table 4) is similar to the uv spectrum of octahydrotrichodimerol (**12**), indicating that the enolized β-diketone systems have remained unchanged.

The ^1H nmr spectrum of **15** (Table 1) displays the signals for hydrogens of methyls (δ 1.33 and 1.49 ppm), an enolized β-diketone hydrogen (δ 16.41 ppm), an acetyl methyl (δ 2.12 ppm), and a methine (δ 3.03 ppm), all as singlets. The ^{13}C nmr spectrum displays 16 carbons corroborating that compound **15** has an element of symmetry. Three carbon absorptions are carbonyl-like (Table 2): one is assigned to the acetate carbonyl (δ 169.0 ppm) and the other two to the enolized β-diketone system (δ 187.5 and 196.3 ppm). The hemiacetal carbon (δ 109.16 ppm) of **15** is shifted with respect to that observed in the ^{13}C nmr spectrum of octahydrotrichodimerol (**12**). From these data, the structure of the diacetate is assigned as the symmetrical hemiacetal diacetate **15**.



15

Triacetate 16.

A fourth product isolated from the acetylation mixture is triacetate **16** with molecular formula, $C_{34}H_{46}O_{11}$, as indicated by the hreims. The molecular ion is confirmed by cims. The ir spectrum of **16** shows absorptions for acetyl carbonyl (1752 cm^{-1}), a conjugated ketone carbonyl (1699 cm^{-1}), a strongly chelated carbonyl (1612 cm^{-1}), but no O-H stretching. The uv spectrum of **16** (Table 4) is more complex than the uv spectrum of octahydrotrichodimerol (**12**) or any of the other acetates (**13**, **14**, or **15**), and indicates that one of the enolized β-diketone systems of **12** has been affected.

The ^{1}H nmr spectrum of **16** (Table 1) shows signals for the acetate methyls (δ 2.11, 2.12, and 2.24 ppm), the quaternary methyls (δ 1.32, 1.35, 1.46, and 1.48 ppm), the methines (δ 3.02 and 3.11 ppm), and the enolized β-diketone hydrogen (δ 16.51 ppm) as singlets, as well as a one hydrogen doublet of doublet of doublets at δ 3.40 ppm (*J* 13, 8.5, 5 Hz) (Table 1). This signal arises from one of the H-8 hydrogens. Interestingly, in the ^{1}H nmr of acetates **13**, **14**, and **15** the signal for the C-8 methylene hydrogens appears as a multiplet of two diastereotopic hydrogens. Although the chemical shift of the C-8 hydrogens is similar in the ^{1}H nmr spectra of compounds **13**, **14**, and **15**, one of the C-8 hydrogens has shifted more than 1 ppm in the ^{1}H nmr spectrum of **16**. This shift demonstrates that the enol system in compound **16** is acetylated.

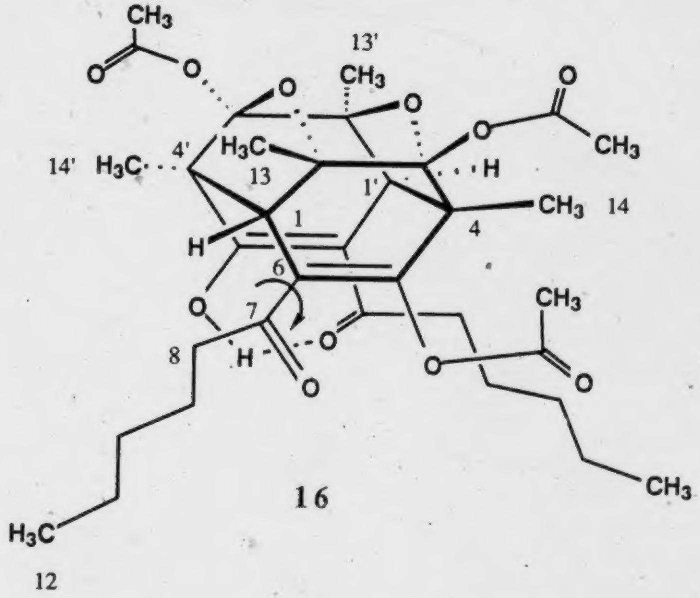
The ^{13}C nmr spectrum of **16** (Table 2) shows the presence of seven carbonyl-like absorptions. Three of these carbon absorptions are attributed to the acetate carbonyls (δ 167.4, 169.2, and 167.2 ppm) and four to the enolized β-diketone and keto-enol carbons (δ 167.4, 188.6, 194.5, and 194.7 ppm).

The ^{13}C nmr spectrum of compound **16** shows shifts for C-5 and C-6 when compared to the ^{13}C nmr of the previous acetates. The oxygen bearing carbon of the acetylated enol (C-5) in **16** has a chemical shift of δ 167.4 ppm while in the previous acetates this carbon appears at δ 187-197 ppm. Similarly the electron-rich carbon of the acetylated enol (C-6) in **16** has a chemical shift of δ 117.5 ppm while in the previous acetates this carbon appears at δ 101-102 ppm.

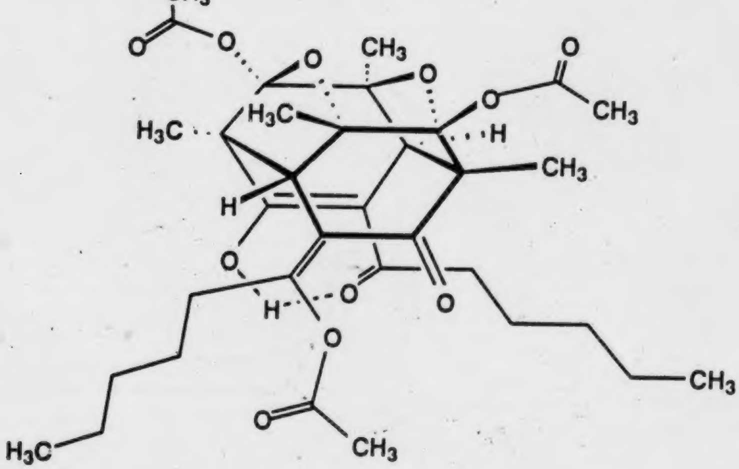
The changes observed in the ^{13}C nmr spectrum of **16** are expected. Acetylation of the enolized β-diketone system changes the electronic nature of the keto-enol system. One of the carbonyl-like carbons becomes a true carbonyl carbon while the other is now an acetylated enolic carbon.

The enol acetate in compound **16** is endocyclic. If compound **16** had an exocyclic enol acetate (as shown in **17**), the side chain would retain its rigidity around the C-6, C-7 bond, and no significant change in the ^{1}H nmr signals of the C-8 hydrogens would be expected. If, on the other hand, the enol acetate of **16** is endocyclic, the absence of chelation would allow extra mobility around the C-6, C-7 bond. This will allow a conformational change of the methylene group that translates into a change in the magnetic environment of the C-8 hydrogens. Since a change has been observed in the ^{1}H nmr of **16** for at least one of the C-8 hydrogens (one of the H-8 hydrogens of **16** has shifted more than 1 ppm as compared to the other acetates), we conclude that the acetylation produced an endocyclic enol acetate.

NOe experiments (Table 3) provide further support for the endocyclic nature of the enol acetate. In nOe enhancement experiments with triacetate **16** the methine at δ 3.02 ppm shows the characteristic nOe enhancement of the C-8 hydrogens (17%). This is similar in magnitude to the enhancements observed in nOe experiments with the monoacetate **13** (10.6 and 11.6%) and the diacetate **15** (27%). However, the other methine of compound **16**, at δ 3.11 ppm shows only a small nOe enhancement (3%) to the C-8 hydrogens. This observation indicates that the C-8 methylene hydrogens are no longer in close proximity to the methine at δ 3.11 ppm which can only be explained by a conformational change in one of the side chains. This observation also establishes the methine at δ 3.11 ppm as H-1, and the methine at δ 3.02 ppm as H-1'.


13'
14'
4'
13
1'
14
1
4
6
7
8
12

16



17

Tetraacetate 18.

A fifth acetate derivative, isolated from the acetylation of [1-^{13}C]acetate labelled octahydrotrichodimerol, is a tetraacetate, **18**, as indicated by the ^{1}H and ^{13}C nmr spectra*. Its ir spectrum shows absorption for acetoxyl carbonyl (1751 cm^{-1}), conjugated carbonyl (1700 cm^{-1}), and double bond (1611 cm^{-1}) groups, consistent with an enol acetate-ketone system.

The ^{1}H nmr spectrum of **18** (Table 1) shows absorptions corresponding to acetyl methyl hydrogens (δ 2.11 and 2.23 ppm), methyl hydrogens (δ 1.30 and 1.45 ppm), a methine hydrogen (δ 3.09 ppm), and a hexanoyl chain {δ 3.10 (m, 1H), 2.50 (m, 1H), 1.45 (m, 2H), 1.28 (m, 4H), and 0.88 (m, 3H) ppm}.

The ^{13}C nmr spectrum of **18** (Table 2) shows 18 signals. As in the case of the symmetrical trichodimerol, which shows 14 signals in its ^{13}C nmr spectrum, the presence of only 18 signals in the ^{13}C nmr spectrum of tetraacetyloctahydrotrichodimerol (**18**) indicates that compound **18** is symmetrical. The signals in the ^{13}C nmr spectrum of **18** correspond to methyl carbons (five), methylene carbons (four), a methine carbon (δ 56.7 ppm), a quaternary carbon (δ 57.8 ppm), a tertiary oxygen bearing carbon (δ 79.1 ppm), an hemiacetal (δ 108.9 ppm), an electron rich sp^2 carbon (δ 117.2 ppm), and several carbonyl like carbons (δ 167.1, 167.5, 169.1, and 193.6 ppm).

The symmetry indicated by the ^{13}C nmr spectrum, the chemical shift of the signals in the ^{13}C nmr spectrum, and the absence of signals attributed to hydroxyl or enolized β-diketone hydrogens in the ^1H nmr spectrum of **18**, indicate that the acetyl groups of **18** are enol acetate and hemiacetal acetates. The ^1H nmr spectrum of **18** indicates that the enol acetates are endocyclic since, as indicated for triacetyloctahydrotrichodimerol (**16**), the chemical shift of the H-8 hydrogens, at δ 2.4-2.5 ppm in the ^1H nmr spectrum of octahydrotrichodimerol, change to δ 3.11 and 2.50 ppm in the ^1H nmr spectrum of tetraacetyloctahydrotrichodimerol (**18**). Therefore, the structure of tetraacetyloctahydrotrichodimerol must be symmetrical as shown in structure **18**.

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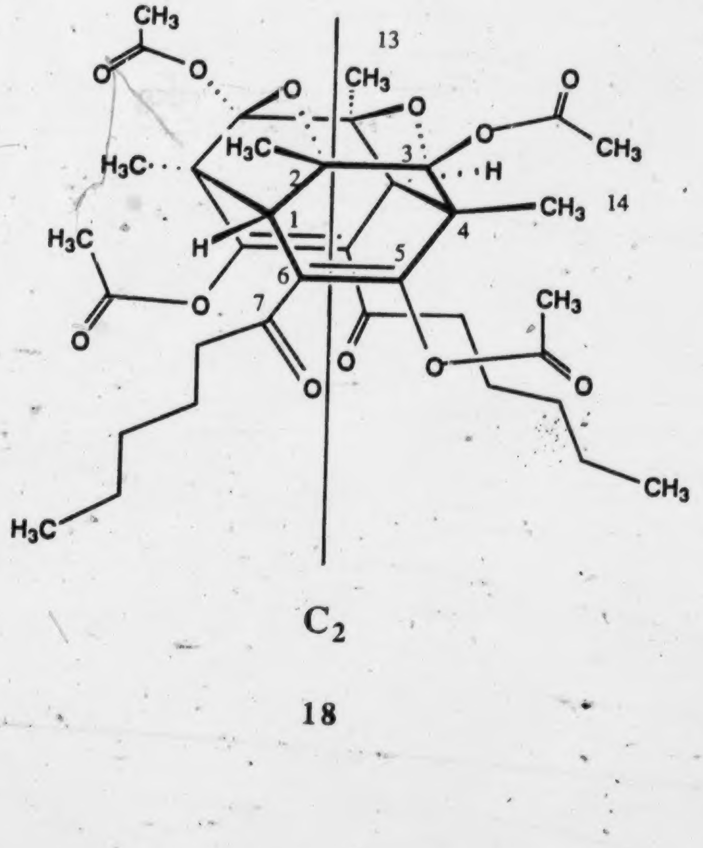
* Since this product was only obtained from a labelled compound, mass spectral data were not obtained.



18

The formation of different acetyl derivatives of octahydrotrichodimerol (**12**) demonstrate its interesting symmetry properties and the presence of an hemiacetal in trichodimerol (**11**). The production of two different monoacetyl derivatives shows the presence of the hemiacetal functionality in trichodimerol. Since octahydrotrichodimerol (**12**) is symmetrical, the progressive introduction of acetyl groups gives the following results: Introduction of one acetyl group, as in monoacetyloctahydrotrichodimerol (**13**) and monoacetyloctahydrotrichodimerol (**14**), destroys the symmetry present in octahydrotrichodimerol (**12**). Introduction of two acetyl groups, as in diacetyloctahydrotrichodimerol (**15**) yields a symmetrical product, while introduction of three acetyl groups, as in triacetyloctahydrotrichodimerol (**16**) destroys the symmetry again. The introduction of four acetyl groups then yields the symmetrical product tetraacetyloctahydrotrichodimerol (**18**).

Table 1. ^{1}H Nmr data of trichodimerol (**11**), octahydrotrichodimerol (**12**), monoacetyloctahydrotrichodimerol (**13**), monoacetyloctahydrotrichodimerol (**14**), diacetyloctahydrotrichodimerol (**15**), triacetyloctahydrotrichodimerol (**16**), and tetraacetyloctahydrotrichodimerol (**18**)[1] ($CDCl_3$, 360 MHz).

	Chemical shifts in δ (ppm[2]), mult., *J* in Hz.						
H	**11**	**12**	**13**	**14**	**15**	**16**	**18**
1	3.00, s	3.00, s	2.98, s^c	3.00, s^c	3.03, s	3.11, s	3.09, s
1'	-	-	3.19, s^c	2.96, s^c	-	3.02, s	-
3 (OH)	3.20, s	3.28, s	6.12, s	2.98, s	-	-	-
5 (OH)	16.33, s	16.66, s	16.23, s^d	16.40, s	16.41, s	-	-
5' (OH)	-	-	15.94, s^d	16.69, s	-	16.51, s	-
8	6.11-6.34, m	2.44-2.52 m	2.25-2.40, m	2.25-2.40, m	2.25-2.40, m	3.40, ddd[3]	3.11, m[4]
8'	-	-	2.25-2.40, m	2.25-2.40, m	-	2.25-2.40 m	-
9	7.32, dd, 15.5,10	1.6, m	1.6, m	1.6, m	1.6, m	1.6, m	1.6, m
10	6.11-6.34, m	1.3, m	1.3, m	1.3, m	1.3, m	1.3, m	1.3, m
11	6.11-6.34, m	1.3, m	1.3, m	1.3, m	1.3, m	1.3, m	1.3, m
12 & 12'	1.89, d,6.5	0.92, t,7	0.85, m	0.85, t,7	0.88, t,7	0.85, m	0.86, t,7
13	1.43, s^a	1.46, s	1.44, s^a	1.43, s^a	1.49, s^a	1.46, s^a	1.45, s^a
13'	-	-	1.47, s^b	1.48, s^b	-	1.48, s^b	-
14	1.46, s^a	1.46, s	1.47, s^b	1.36, s^b	1.33, s^a	1.314, s^b	1.31, s^a
14'	-	-	1.51, s^a	1.41, s^a	-	1.35, s^a	-
$COC\underline{H}_3$	-	-	2.03, s	2.11, s	2.12, s	2.12, s	2.11, s
$COC\underline{H}_3$'	-	-	-	-	-	2.12, s	-
$COC\underline{H}_3$"	-	-	-	-	-	2.24, s	2.23, s

[1] Signals with the same superscript in the same column are interchangeable. [2] Relative to the residual solvent signal (7.262 for chloroform). [3] ddd, J = 13.5, 8.5, 5 Hz. [4] The other H-8 signal is in the multiplet at 2.40 ppm.

Table 2. ^{13}C Nmr data of trichodimerol (**11**), octahydrotrichodimerol (**12**), monoacetyloctahydrotrichodimerol (**13**), monoacetyloctahydrotrichodimerol (**14**), diacetyloctahydrotrichodimerol (**15**), triacetyloctahydrotrichodimerol (**16**), and tetraacetyloctahydrotrichodimerol (**18**) ($CDCl_3$,75.5 MHz).

	Chemical shift in δ (ppm^{1,2})						
C	**11**^{3,4}	**12**^{3,4}	**13**^{3}	**14**^{4}	**15**^{3,4}	**16**^{3,4}	**18**^{5}
1	57.6	57.3	53.3^{a}	58.0^{a}	57.7	56.7	56.7
1'	-	-	56.8^{a}	57.5^{a}	-	57.5	-
2	78.9	78.8	89.0	78.9^{e}	78.9	79.1^{d}	79.1
2'	-	-	78.4	78.6^{e}	-	78.7^{d}	-
3	104.1	103.9	104.4	109.5	109.2	108.7	108.9
3'	-	-	202.2	103.5	-	109.1	-
4	58.9	58.0	60.8^{b}	58.6	58.6	60.8	57.8
4'	-	-	63.8^{b}	58.0	-	58.5	-
5	197.9	193.4	196.5^{c}	187.3	187.5	167.4^{c}	167.5
5'	-	-	195.1^{c}	193.5^{b}	-	188.6	-
6	102.8	103.3	106.0^{g}	102.0^{c}	101.4	117.5	117.2
6'	-	-	106.4^{g}	102.7^{c}	-	101.0	-
7	176.0	191.9	188.4	196.5^{b}	196.3	194.5^{a}	193.6
7'	-	-	188.4	191.8^{b}	-	194.7^{a}	-
8	118.6	34.7	33.6	33.3	33.4	33.7	33.3
8'	-	-	33.7	34.7	-	32.9	-
9	143.6	25.2	25.0^{d}	25.8	25.8	26.8	26.6
9'	-	-	25.9^{d}	25.1	-	25.3	-
10	131.0	31.5	31.6	31.6	31.6	31.4	31.8
10'	-	-	31.7	31.5	-	31.6	-
11	140.3	22.4	22.4^{e}	22.4	22.4	22.4	22.4
11'	-	-	23.8^{e}	22.4	-	22.4	-
12	18.7	13.9	13.9	13.9	13.9	13.9	13.9
12'	-	-	13.9	13.9	-	13.9	-
13	21.3	21.2	23.8^{f}	21.5^{d}	21.4^{a}	21.3^{b}	21.3^{a}
13'	-	-	22.4^{f}	21.1^{d}	-	21.4^{b}	-
14	18.9	18.8	21.4^{f}	20.3^{d}	20.2^{a}	20.4^{b}	20.2^{a}
14'	-	-	20.0^{f}	18.6^{d}	-	20.2^{b}	-
CO	-	-	173.3	169.0	169.0	169.2	169.1
Me	-	-	20.3^{f}	?	20.7^{a}	20.6^{b}	20.7^{a}
CO	-	-	-	-	-	167.2^{c}	167.1^{b}
Me	-	-	-	-	-	21.0^{b}	20.9^{a}
CO	-	-	-	-	-	167.4^{c}	-
Me	-	-	-	-	-	21.1^{b}	-

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[1] Signals with the same superscript in the same column are interchangeable. [2] Relative to the carbon of the solvent (77.0 for chloroform). [3] APT obtained. [4] Also obtained from [1-^{13}C]acetate incorporated trichodimerol. [5] Only obtained from [1-^{13}C]acetate incorporated trichodimerol.

Table 3. ^{1}H Nmr nOe experiments with trichodimerol (**11**), octahydrotrichodimerol (**12**), monoacetyloctahydrotrichodimerol (**13**), monoacetyloctahydrotrichodimerol (**14**), diacetyloctahydrotrichodimerol (**15**), and triacetyloctahydrotrichodimerol (**16**) ($CDCl_3$, 360 MHz).

Signal saturated	Signal enhanced (%nOe[a])
11	
1.43 (H-13)	3.00 (H-1) (2.8)
1.46 (H-14)	3.00 (H-1) (3.2)
	6.12 (H-8) (1.0)
3.00 (H-1)	1.43 (H-13) (5)
	1.46 (H-14) (7.8)
	6.12 (H-8) (16)
12	
1.46 (H's-13, 14)	2.44-2.52 ((H-8's) (0.6)
	3.00 (H-1) (3.9)
	3.28 (OH-3) (1.6)
2.44 (H-8a)	3.00 (H-1) (5.1)
2.52 (H-8b)	3.00 (H-1) (5.7)
3.00 (H-1)	2.44-2.52 (H-8's) (12)
	1.46 (H-13 & 14) (10)
3.28 (OH-3)	1.46 (H-13 & 14) (7.4)
13	
1.44 (H-13)	2.98 (H-1) (3.7)
	6.12 (OH-3) (12)
1.47 (H-14, H-13')	3.19 (H-1') (4.5)
	6.12 (OH-3) (1.3)
1.51 (H-14')	2.38 (H-8's) (0.9)
	2.98 (H-1) (5.2)
2.98 (H-1)	1.44 (H-13) (6.4)
	1.51 (H-14') (5.3)
	2.38 (H-8's) (10.6)
3.19 (H-1')	1.469 & 1.474 (H-13' & H-14) (11.1)
	2.31 (H-8') (11.6)
6.12 (OH-3)	1.44 (H-13) (9.3)
14	
1.36 (H-14)	2.96 (H-1') (4)
1.41 (H-14')	3.00 (H-1) (6.2)
1.43 (H-13)	3.00 (H-1) (3.4)
1.48 (H-13')	2.96 (H-1') (2.5)
15	
1.33 (H-14)	3.03 (H-1) (9.6)
1.49 (H-13)	3.03 (H-1) (11)
3.03 (H-1)	1.33 (H-14) (5.3)
	1.49 (H-13) (3.3)
	2.35 (H-8) (27)
16	
1.32 (H-14)	3.02 (H-1') (1.2)
	2.30 (H-8) (2)
1.35 (H-14')	3.11 (H-1) (2.2)
1.46 (H-13)	3.11 (H-1) (5.7)
1.48 (H-13')	3.02 (H-1') (3.7)
3.02 (H-1')	1.32 (H-14) (6)
	1.48 (H-13') (5)
	2.30 (H-8) (17)
3.11 (H-1)	1.35 (H-14') (7.6)
	1.46 (H-13) (4)
	2.30 (3)
3.40 (H-8a)	2.30 (H-8b) (24)

[a] Ratio of the enhanced area over the irradiated signal's area in the difference spectra.

Table 4. Uv data of trichodimerol (**11**), octahydrotrichodimerol (**12**), monoacetyloctahydrotrichodimerol (**13**), monoacetyloctahydrotrichodimerol (**14**), diacetyloctahydrotrichodimerol (**15**), and triacetyloctahydrotrichodimerol (**16**) (MeOH).

Compound	λ_{max} nm (ε)[a]	λ_{max} nm (ε) in base[b]
11	240 (10000), 295 (15500), 307 (15800), 362 (30000)	378
12	294 (14000), 340 (390)	-
13	295 (12000)	203 (34000), 307 (12000)
14	292 (16000)	204 (49000), 308 (16000)
15	290 (20000)	204 (55000), 299 (22000)
16	224 (8000), 229 (8000), 255 (12000), 292 (10000)	205 (78000), 248 (10000) 304 (18000)

[a] In neutral solution. [b] Upon addition of a drop of 5% sodium hydroxide. In all cases acid regenerated the original spectrum.

Absolute stereochemistry of trichodimerol (11).

The circular dichroism (CD) spectrum of trichodimerol (**11**) is very interesting. It shows two strong Cotton effects of opposite sign (amplitude (A) = $\Delta\varepsilon_1 - \Delta\varepsilon_2 = -25$) as shown in Figure 3. This type of pattern is known as a split Cotton effect (52).

The split Cotton effect can be used to establish the absolute stereochemistry of trichodimerol since it contains information on the relative stereochemical arrangement of the two chromophores*. A split Cotton effect pattern is typical of a two chromophore system wherein two "inherently symmetric" chromophores are "dissymmetrically" located respective to one another (54). This gives rise to the so-called "exciton chirality method" or more specifically the "dibenzoate rule" (when benzoates are involved). The identical chromophores in trichodimerol are inherently symmetric since they are planar. This, together with the fact that the CD spectrum shows a split Cotton effect indicates that the two chromophores in trichodimerol must be dissymmetrically located respective to one other**.

The coupling between the electric transition dipole moment vectors of the chromophores is the source of the split Cotton effect. Therefore a knowledge of the orientation of the electric transition dipole moment vector in the chromophore is necessary in order to use the exciton chirality method to assign the absolute stereochemistry. The arrangement of the electric transition dipole moment vectors is characterized by their "screwness". A "left handed screwness" gives rise to a "negative chirality", while a "right handed screwness" gives rise to a "positive chirality" (Figure 4).

---

* "Since the chiral exciton coupling method is based on sound theoretical calculations,..., the absolute stereochemistry of organic compounds exhibiting typical split CD Cotton effects due to chiral exciton coupling is assignable in a non-empirical manner." (53)

** The simple fact that the CD shows a split Cotton effect demonstrates the dimeric nature of trichodimerol.

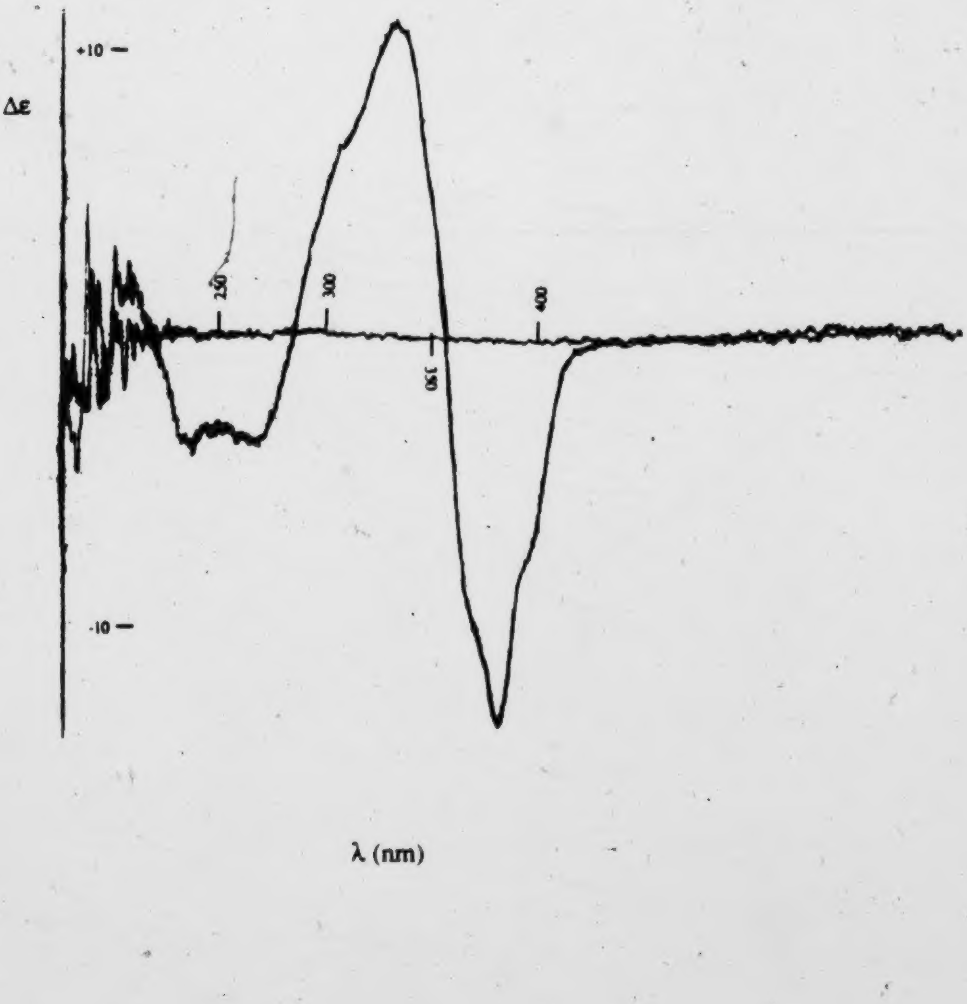

Figure 3. CD spectrum of trichodimerol (11) (MeOH).

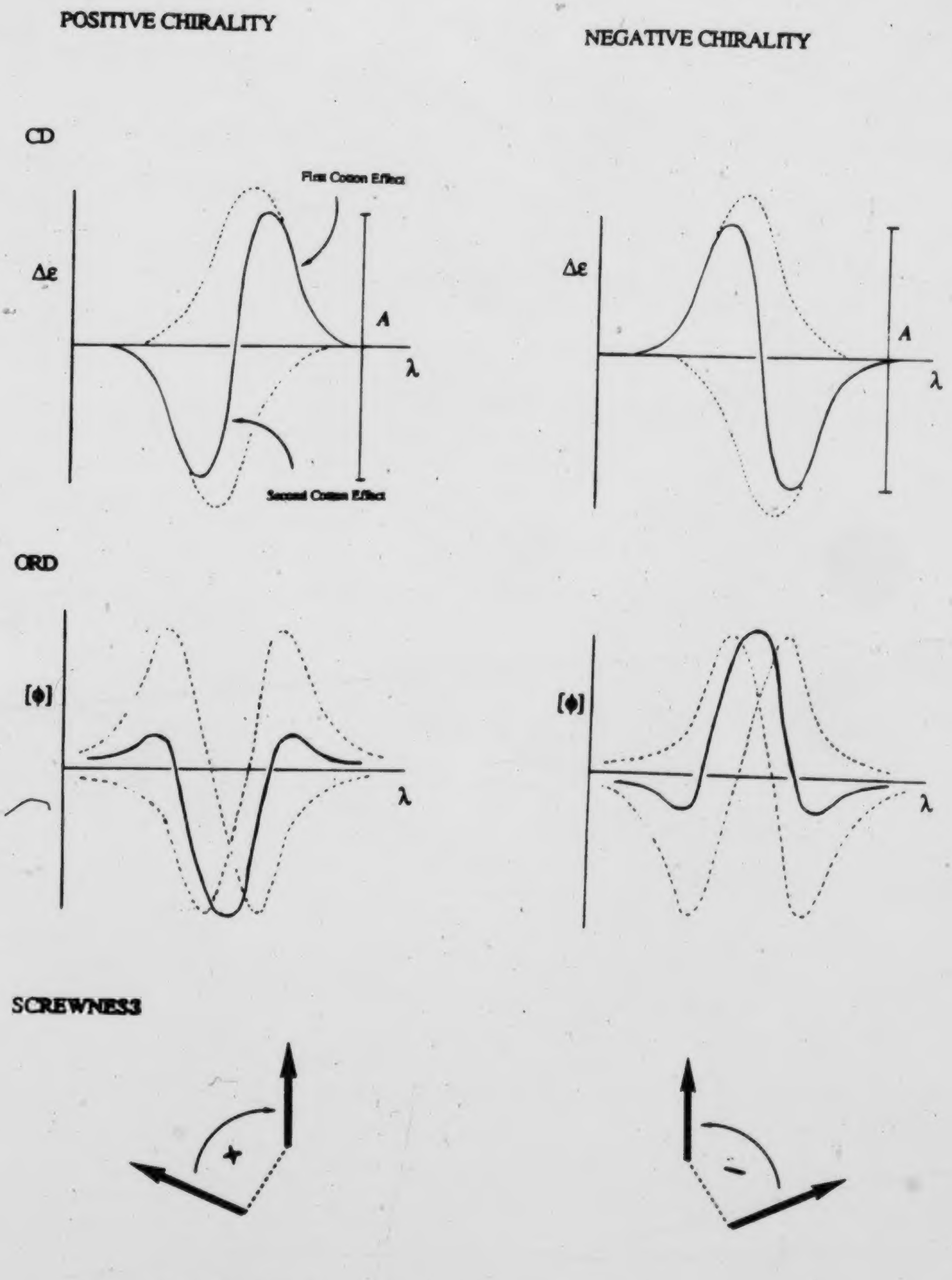
POSITIVE CHIRALITY
NEGATIVE CHIRALITY
CD
First Cotton Effect
Δε
A
λ
Second Cotton Effect
Δε
A
λ
ORD
[ϕ]
λ
[ϕ]
λ
SCREWNESS
+
−

Figure 4. Typical ORD and CD spectra of coupled chromophore systems.
(Adapted from reference 52)

A positive or negative chirality can be recognized by the sign of the long wavelength Cotton effect*. Negative chiralities have a negative first Cotton effect and positive chiralities have a positive first Cotton effect. The first Cotton effect of the CD spectrum of trichodimerol (**11**) is negative and followed by a positive second Cotton effect, indicating that trichodimerol has a negative chirality. Since it is safe to assume that the electric transition dipole moment vector of the chromophores in trichodimerol lies along the long axis of the two unsaturated systems, we can conclude that the absolute stereochemistry of trichodimerol is as shown in **11**.

The optical rotatory dispersion (ORD) spectrum of coupled chromophores has a characteristic shape. The shape of the ORD curve in coupled chromophores is the summation of two Cotton effects of different sign, as seen in Figure 4. As can be seen in Figure 5, the ORD spectrum of trichodimerol resembles the expected curve for a negative chirality. This observation confirms that the chromophores in trichodimerol are coupled with a negative chirality confirming that the absolute stereochemistry of trichodimerol is as shown in **11**.

Care must be taken when interpreting CD and ORD spectra since the chirality of chromophore systems depends on the orientation of the electric transition dipole moment vectors of the chromophores in consideration. For example, examination of the chiroptical properties of octahydrotrichodimerol (**12**) indicates that octahydrotrichodimerol has a

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* The extremum at longer and shorter wavelengths are called first and second Cotton effects respectively.

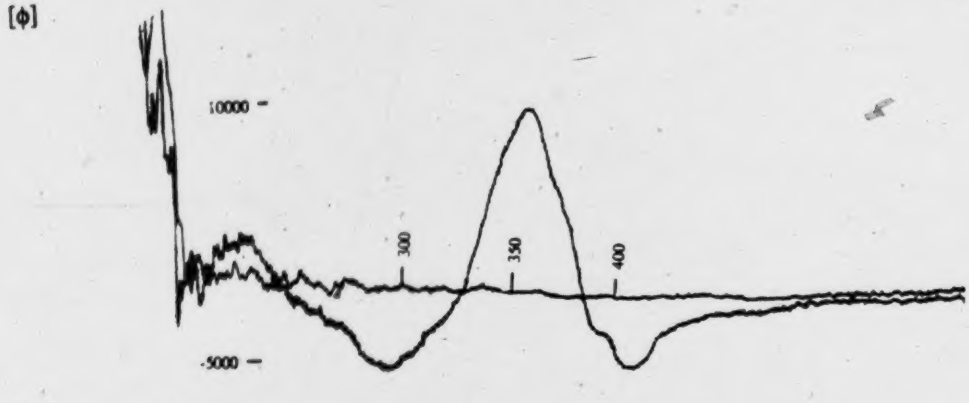


Figure 5. ORD spectrum of trichodimerol (11) (MeOH).

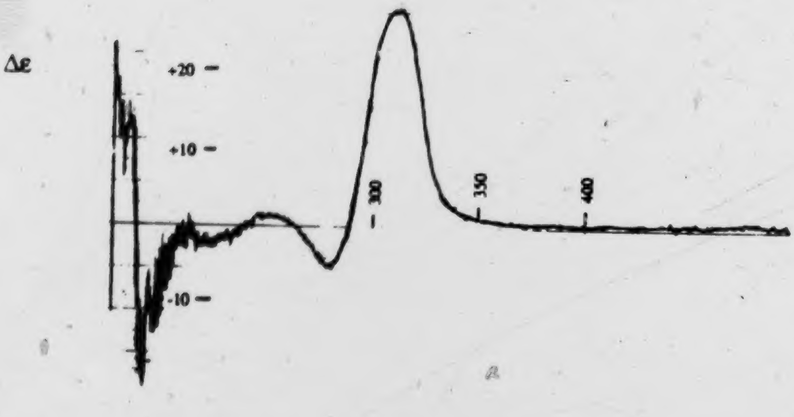


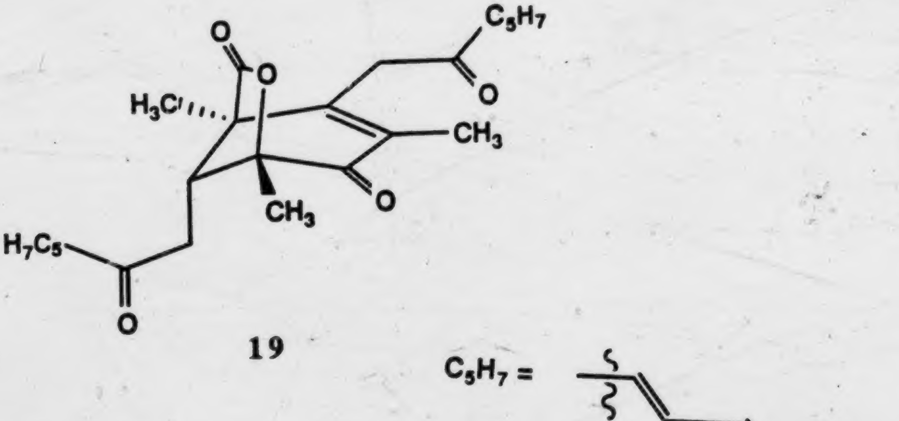
Figure 6. CD spectrum of octahydrotrichodimerol (12) (MeOH).

positive chirality. The CD spectrum of octahydrotrichodimerol (**12**) (Figure 6) shows a split Cotton effect with extrema at 311 and 281 nm (*A* = +33). This observation might lead to the conclusion that the absolute stereochemistry of octahydrotrichodimerol, and hence trichodimerol, is antipodal to that shown in **12**. However this cannot be the case. Since we do not know the direction of the electric transition dipole moment vector of the enolized β-diketone system, analysis of the absolute stereochemistry of octahydrotrichodimerol by use of the exciton coupling method might have led us to derive a wrong absolute stereochemistry for trichodimerol. The change in chirality between trichodimerol and octahydrotrichodimerol indicates that the electric transition dipole moment vector of octahydrotrichodimerol (**12**) changed direction when compared to the electric transition dipole moment vector of trichodimerol (**11**). Such a change in the chromophore explains a coupling of different sign.

Trichodermolide (19)

A second unusual polyketide isolated from the mycelium of *T. longibrachiatum* has been named trichodermolide. It is a pale yellow, optically active {$[\alpha]_D$ +97°(*c* 0.97, EtOH)} solid. The molecular formula, $C_{24}H_{28}O_5$, was obtained from the hreims and confirmed by cims. Its uv spectrum is different from that of trichodimerol, showing maxima at 280 (log ε = 4.72) and 217 (4.15) nm, which is unchanged on addition of base or acid, thus indicating that the chromophores present do not have either basic or acidic properties.

The ir spectrum of trichodermolide shows the presence of a γ-lactone (1781 cm^{-1}) and an α,β-unsaturated ketone (1687 and 1637 cm^{-1}).



19

Most peaks in the hreims are of relatively low intensity due to the intensity of the base peak, at m/z 95 (C_6H_7O), which is characteristic of a sorbyl chain in this series of compounds. In addition the hreims shows fragment ions at m/z 301, due to the loss of the sorbyl chain, and at m/z 352, due to the loss of carbon dioxide. The fragment ion at m/z 352 supports the presence of a lactone, as inferred from the ir spectrum.

The ^{1}H nmr spectrum of trichodermolide (**19**) (Table 5) shows no exchangeable hydrogens*. It shows signals attributed to methyls (δ 1.78, 1.45, and 1.24 ppm) as singlets, an AB system (δ 3.66 ppm), two sorbyl chains, and a three hydrogen spin system {δ 2.45 (dd, 18.5, 4 Hz, 1H), 3.26 (dd, 18.5, 6 Hz, 1H), and 3.34 (dd, 4, 6 Hz, 1H) ppm}.

When the ^{1}H nmr spectrum of trichodermolide is recorded in benzene the methylene, at δ 3.66 ppm in $CDCl_3$, splits into two signals at δ 3.02 and 3.19 ppm with a coupling constant of 17 hertz. This large coupling constant suggests that this methylene is adjacent to a carbonyl*. Furthermore, the large geminal coupling constant between the hydrogens at δ 2.45 and 3.26 ppm (18.5 Hz) indicates another methylene adjacent to a carbonyl.

The ^{13}C nmr spectrum of trichodermolide (**19**) (Table 6) shows 24 signals, assigned to five methyl carbons, two methylene carbons, a methine carbon (δ 56.0 ppm), a quaternary carbon (δ 51.8 ppm), a tertiary carbon bearing oxygen (δ 87.2 ppm), eight sp^2 carbons as doublets, sp^2 carbons as singlets (δ 134.2 and 148.6 ppm), and several carbonyl carbons (δ 176.1, 191.2, 195.5, and 196.63 ppm).

The signal at δ 87.2 ppm in the ^{13}C nmr spectrum must be part of the lactone since it is low field for an oxygen bearing sp^3 carbon. Esters of tertiary alcohols are shifted downfield, relative to the respective alcohols, by about 10 ppm (56).

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* No signal disappeared upon addition of D_2O.

* The geminal coupling constants of groups adjacent to double bonds is larger in magnitude (17-22 Hz)than the coupling constant of other alicyclic methylenes (usually 13-14 Hz) (55).

The molecular formula of trichodermolide indicates it has eleven unsaturations. Two sorbyl chains account for six unsaturations, the lactone for two, and the α,β-unsaturated ketone for two, suggesting the presence of a single carbocyclic ring. The two sorbyl chains, the lactone, and the α,β-unsaturated ketone account for all of the oxygens.

In order to obtain further information on the structure of trichodermolide (**19**) a series of nOe experiments was performed (Table 7). The methine hydrogen at δ 3.34 ppm shows strong nOe enhancement with the methylene hydrogen at δ 2.45 ppm and with the methyl hydrogens at δ 1.45 and 1.24 ppm. The methyl hydrogens at δ 1.24 ppm also show nOe enhancement with the methylene hydrogen at δ 3.26 ppm and with the methylene signal at δ 3.66 ppm. The methylene at δ 3.66 ppm in turn gives enhancements with the methyl hydrogens at δ 1.78 ppm and to the hydrogen signals from one of the sorbyl chains at δ 6.15 and 7.24 ppm. The hydrogens at δ 3.26 and 2.45 ppm give strong mutual enhancements (as expected for geminal hydrogens) and enhancement to hydrogens from the other sorbyl chain at δ 6.10 and 7.20 ppm. These results and others are summarized in Figure 7.

Furthermore, the nOe enhancement observed between the methylene hydrogens at δ 2.45 and 3.26 ppm and hydrogens of a sorbyl chain indicates that this methylene is close to one of the sorbyl chains, while the nOe enhancement observed between the methylene at δ 3.66 ppm and the hydrogens of a sorbyl chain indicate that this methylene is located close to the other sorbyl chain.

The methyl at δ 1.78 ppm, which is alkenic and on the α carbon of the α,β-unsaturated ketone on the basis of its ^1H nmr chemical shift (57), shows nOe enhancement with the methylene at δ 3.66 ppm. Therefore, this methylene is also alkenic and on the β carbon of the α,β-unsaturated ketone*. These data allow for the presence of substructure **20** in trichodermolide. A further fragment, substructure **21**, is suggested by the nOe enhancements observed for the methine at δ 3.34 ppm. Substructures **20** and **21** may be combined to form partial structure **22**, which may be completed by inclusion of the lactone group. At this stage two structures were possible for trichodermolide. They differ in the orientation of the lactone carbonyl. The lactone oxygen may have a 1,3 relationship to the α,β-unsaturated ketone, as shown in structure **23**, or a 1,5, as shown in structure **24**.

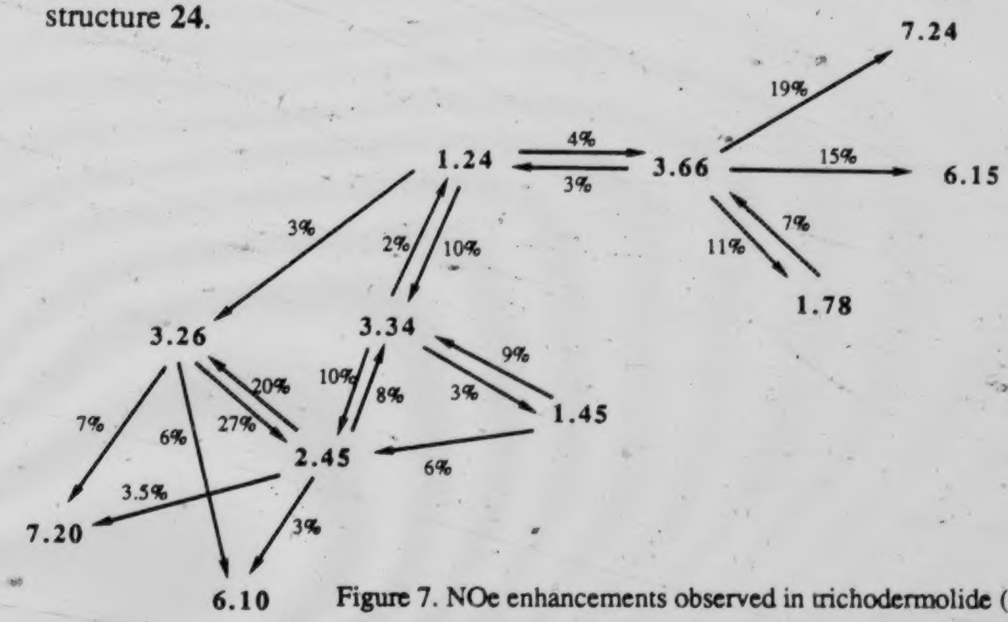

Figure 7. NOe enhancements observed in trichodermolide (**19**) (acetone-d_6, 360 MHz).

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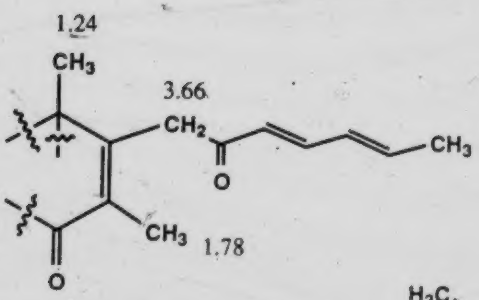
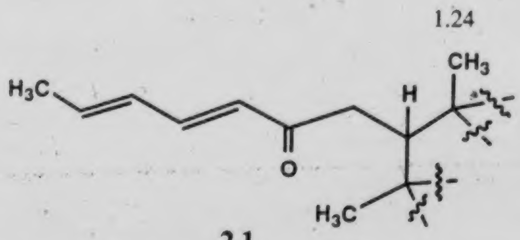
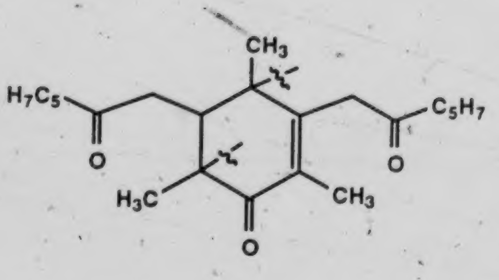
* Had the methylene at δ 3.66 ppm been on the carbon α to the carbonyl, there is no possibility it would show this nOe, and the chemical shift of the methyl would be around δ 1.96 ppm (57).


1.24
CH3
3.66
CH2
O
CH3
O
CH3
1.78

20

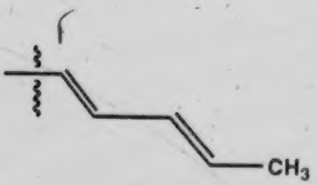

1.24
CH3
H3C
H
O
H3C
1.45

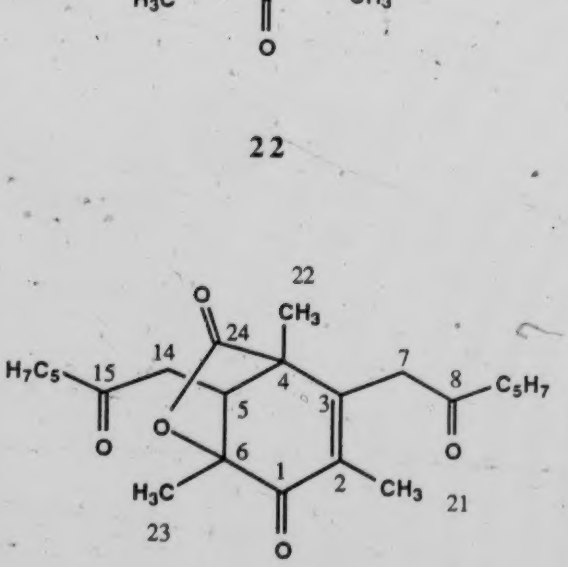
21


CH3
H7C5
C5H7
O
O
H3C
CH3
O

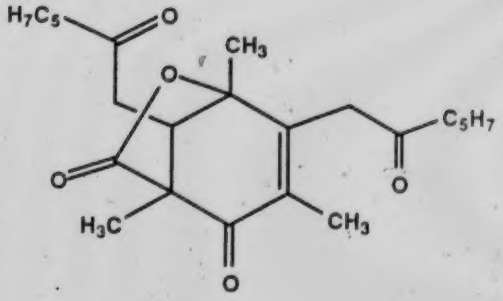
22

C_5H_7 =




22
O
24
CH3
H7C5
14
15
7
8
C5H7
4
3
5
O
O
O
6
1
2
H3C
CH3
21
23
O

23


H7C5
O
CH3
O
C5H7
O
O
H3C
CH3
O

24

The ^{1}H nmr data do not allow differentiation between structures **23** and **24** for trichodermolide. The methyl signal at δ 1.45 ppm could arise from a methyl on a carbon bearing an oxygen as in **23**, or from a methyl group adjacent to two carbonyls, as in **24**. In order to differentiate between structures **23** and **24** we made use of the selective INEPT* technique (58).

The potential of the selective INEPT technique has been available since the design of the original refocused INEPT experiment by Morris and Freeman (59). Recently there has been an "explosion" in the use of the selective INEPT technique in structure elucidation and ^{13}C nmr spectral assignment (60). In a selective INEPT experiment, the basic refocussed INEPT with hydrogen decoupling sequence is used with an initial soft, selective pulse on hydrogen while the delays are maximized for long range H-C couplings. This experiment gives signals for the carbons that are long range coupled to the irradiated hydrogen, all other signals are eliminated by the phase cycle.

The selective INEPT technique has a disadvantage. The one bond coupling H-C gives rise to a "passive" modulation of the intensity of the observed signal. For this reason, hydrogen-bearing carbons (61) which are long range coupled to the selectively irradiated hydrogen might not give a signal. Martin and Zektzer (62) have discussed this passive modulation in the context of two dimensional (2D) experiments. Their arguments apply to the one dimensional (1D) technique since the 2D techniques discussed by them use INEPT-based sequences (62).

The results from the selective INEPT experiments on trichodermolide (Figure 8) show that the structure of trichodermolide is **23**.

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* Also known as INAPT.

Selective irradiation of the methyl hydrogens at δ 1.24 ppm gives signals for the carbons at δ 51.7, 176.1, and 149.6 ppm. Irradiation of the methyl hydrogens at δ 1.45 ppm gives signals for the carbons at δ 86.9 and 191.2 ppm. These experiments assign the methyl at δ 1.24 ppm as Me-22 and at δ 1.45 ppm as Me-23. If the structure of trichodermolide was **24** it would be impossible for both methyls to give carbonyl signals.

The carbon at δ 55.6 (d) ppm (C-5) was not enhanced in either experiment although it should be coupled ($^3J_{CH}$) to both methyls. The absence of enhancement may be due to the passive modulation of the carbon at δ 55.6 ppm by the one bond H-C coupling.

Although the structure of trichodermolide was established as **23** the relative stereochemistry of C-5 could not be unambiguously assigned at this stage. The two possible configurations at C-5 could give the observed nOe enhancements. Therefore we required further chemical information, which we obtained from the production of several derivatives as follows.

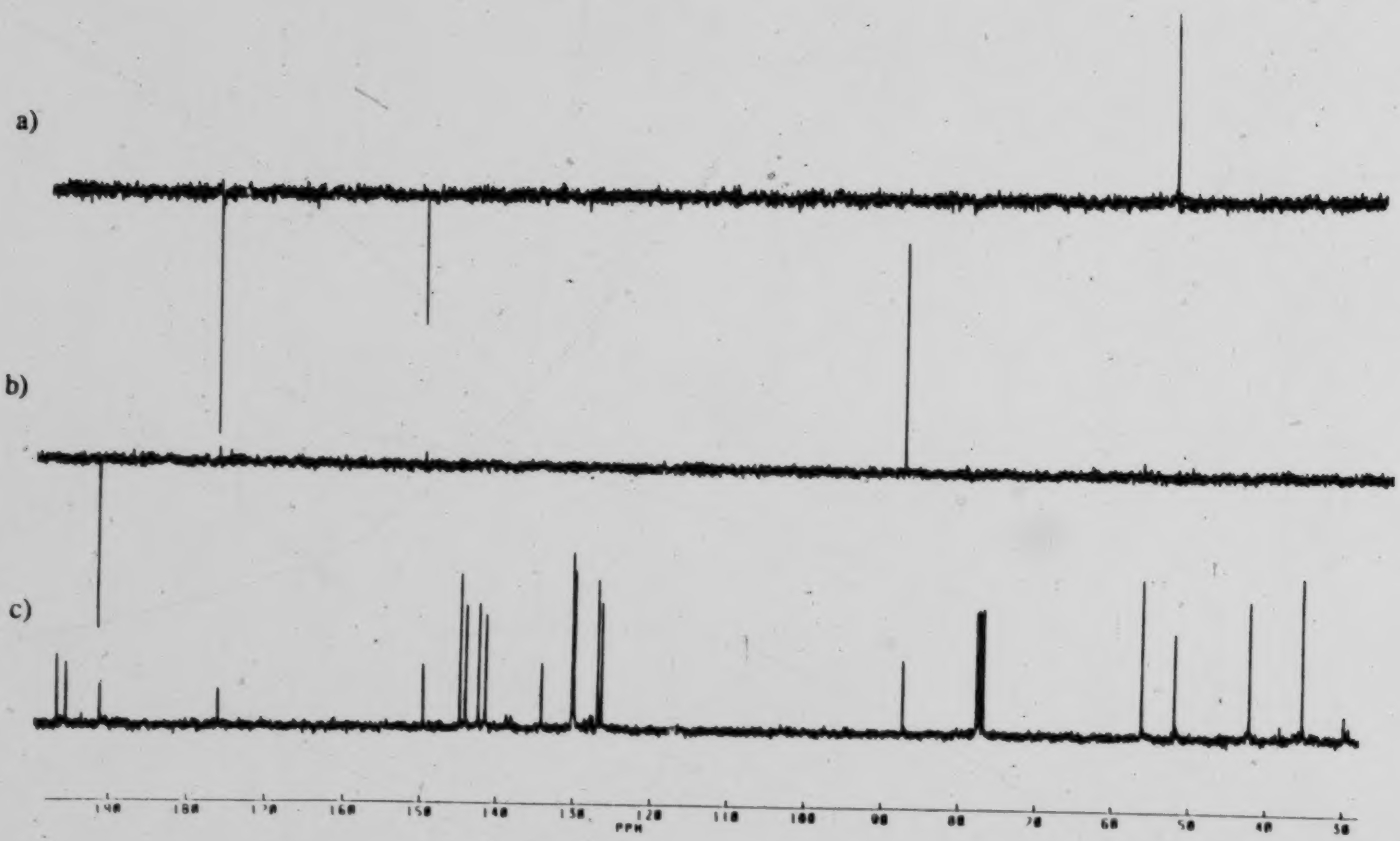

Figure 8. Selective INEPT experiments with trichodermolide (**19**) ($CDCl_3$, 75.5 MHz) a) Selective irradiation at δ 1.24 ppm. b) selective irradiation at δ 1.45 ppm. c) Broad band ^{13}C nmr spectrum of trichodermolide (**19**).

Hydrogenation of trichodermolide (19).

An octahydro derivative of trichodermolide is obtained by catalytic hydrogenation of trichodermolide over palladium on carbon. The molecular formula, $C_{24}H_{36}O_5$, was obtained from the hreims and confirmed by chemical ionization mass spectrometry. The mass spectrum of octahydrotrichodermolide (**25**) shows fragment ions for the loss of a hexanoyl group at m/z 306 and the subsequent loss of CO_2 at m/z 262. The ir spectrum of **25** shows the presence of lactone (1782 cm^{-1}), ketone (1715 cm^{-1}), and α,β-unsaturated ketone (1688 cm^{-1}) groups. The uv spectrum of **25** confirms the presence of an α,β-unsaturated ketone, with maxima at 219 ($\varepsilon = 5600$), 272 (5700), 325 (1200), and 329 (1200) nm.

The ^1H nmr spectrum of octahydrotrichodermolide (**25**) (Table 5) is assigned with the aid of nOe experiments (Table 7). Comparison of the ^1H nmr chemical shifts of **25** with those of **19** reveals that the C-14 methylene hydrogens shift upfield from δ 3.26 and 2.45 ppm in **19** to δ 3.12 and 2.30 ppm (0.14 and 0.15 ppm) in **25** and the C-7 methylene hydrogens from δ 3.66 in **19** to δ 3.48 ppm (0.18 ppm) in **25**. The observed shifts are consistent with these groups being adjacent to the sorbyl chains.

The ^{13}C spectrum of **25** is summarized in Table 6. Comparison of the ^{13}C nmr spectra of **25** and **19** shows that the most significant differences are the shift of the C-7 and C-14 signals (δ 35.0 and 41.9 ppm, downfield by 2.1 and 2.5 ppm, respectively).

Sodium borohydride reduction of octahydrotrichodermolide.

In order to obtain further support for the structure of trichodermolide we investigated the sodium borohydride reduction of octahydrotrichodermolide. The reduction of octahydrotrichodermolide with excess sodium borohydride at room temperature (5 min.) in methanol gave a complex mixture of products. In an attempt to obtain higher selectivity, the reduction procedure was carried out in methanol/benzene at 0°C for a couple of minutes. Under these conditions a mixture of two inseparable products was usually obtained. However, on one occasion only one product was obtained. Subsequently it was found that an epimeric mixture of alcohols was formed. Acid treatment of the mixture of alcohols resulted in dehydration of only one of the epimers, thereby allowing the isolation of the minor isomer. This allowed the spectral characterization of both products to be determined (see Experimental). The structure of the products was determined in the following way:

Neither the major nor the minor isomer show strong uv absorption in their uv spectra indicating that in each isomer the α,β-unsaturated ketone was reduced. The ir spectrum of each isomer shows absorption for an alcohol (3460 and 3450 cm^{-1}) while the ^1H nmr spectrum (Table 5) of each isomer shows signals attributable to the presence of two hexanoyl side chains.

In a ^1H nmr experiment on the major isomer, the methyls at δ 1.46 and 1.69 ppm gave nOe enhancements to the alcoholic methine hydrogen, at δ 3.66 ppm* (Table 7). This indicates that these methyl groups are on carbons α to the alcohol in the major isomer and, therefore, confirm these methyls are on carbons α to the α,β-unsaturated ketone in trichodermolide (**19**) (at δ 1.45 and 1.78 ppm in the ^{1}H nmr of trichodermolide).

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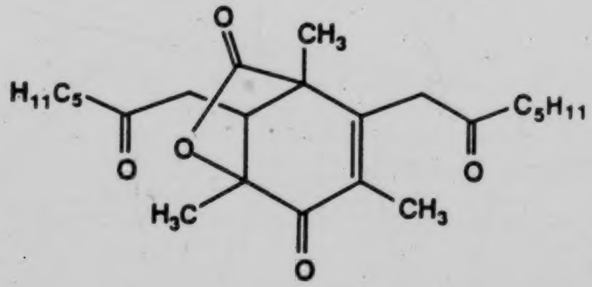
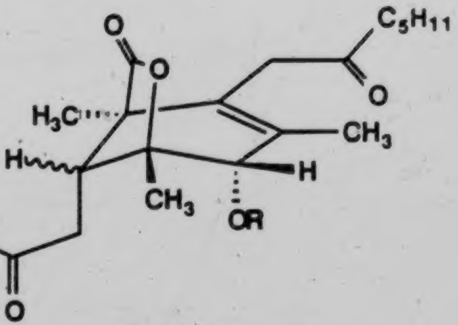
* The nOe experiments were performed on the pure product.

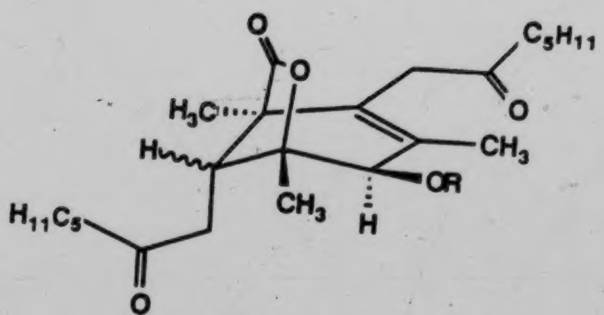
The reasons for the regiochemical selectivity of the reduction under the reaction conditions are not known. This result is, however, not surprising since six membered α,β-unsaturated ketones are particularly reactive towards sodium borohydride (63).



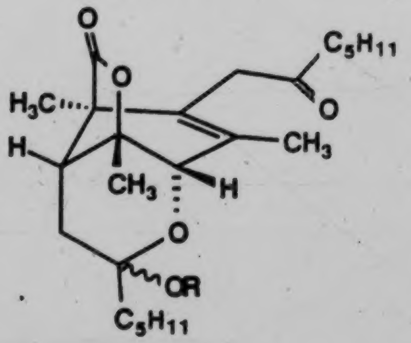
25



27a R= H
27b R= Ac



28a R= H
28b R= Ac



29a R = Me
29b R = H

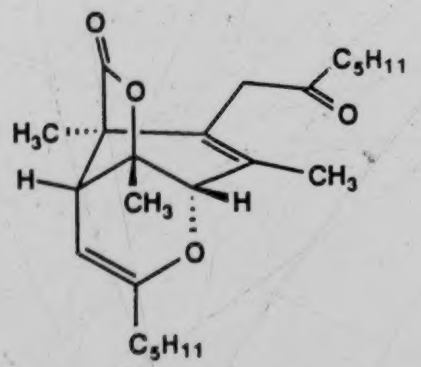
Acid catalyzed formation of anhydrodecahydrotrichodermolide (26) and its subsequent aromatization.

At this point the two epimers (**27a** or **28a**) cannot be distinguished. The relative stereochemistry of the two was investigated in the following way:

It was assumed that if the hydrogen on C-5 was β, the α epimer (**27a**) should be able to form an hemiacetal with the ketone of one of the side chains, to give **29b**, while the β isomer should not. Accordingly, the major product was treated with p-toluensulphonic acid in methanol in order to obtain **29a**. Monitoring the reaction by tlc revealed an initial product (**29b**?) was soon replaced by a second compound, which proved to be anhydrodecahydrotrichodermolide (**26**), allowing us to establish that the major alcohol is the α isomer, **27a** and establishing the relative stereochemistry at C-5. Therefore the structure of trichodermolide should be as shown in **19**.

The structure of anhydrodecahydrotrichodermolide (**26**) was established as follows. The molecular formula, $C_{24}H_{36}O_4$, was obtained from the hreims and confirmed by cims. The ir spectrum showed absorption for lactone (1779 cm^{-1}) and ketone (1715 cm^{-1}) groups. Comparison of the ^{13}C nmr of **26** with that of **27a** reveals that **26** has an additional double bond (Table 6). The chemical shift of the alkenic carbons of this double bond indicates it is part of an enol ether (δ 92.9 and 153.8 ppm) (64). Furthermore, comparison of the ^1H nmr spectrum of **26** with the ^1H nmr spectrum of α-decahydrotrichodermolide (**27a**) shows that the three hydrogen spin system in **27a** has been replaced by mutually coupled hydrogens at δ 4.56 and at δ 2.19 ppm (6 Hz) in **26**. From these data we conclude that the enol ether in **26** is endocyclic. The C-1 methine in **27a** is shifted from δ 3.71 to 4.08 ppm (0.37 ppm) in **26**.

The COSY (65) spectrum of anhydrodecahydrotrichodermolide (**26**) (Figure 9) shows several interesting correlations. The methyl at δ 1.68 ppm (Me-21) correlates with the methylene at δ 3.21 ppm (H-7) and the



26

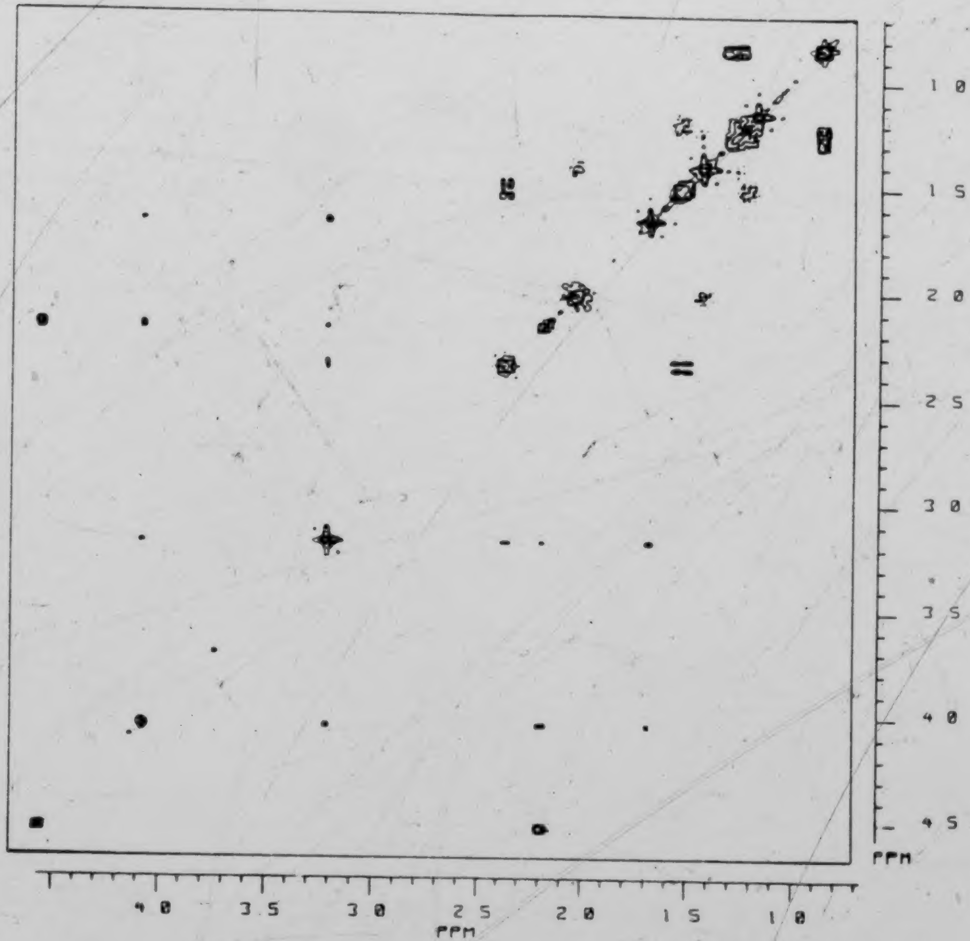


Figure 9. COSY 90 spectrum of anhydrodecahydrotrichodermolide (26) (CDCl$_3$, 360 MHz).

methine at δ 4.08 ppm (H-1), while the methylene at δ 3.21 ppm (H-7) correlates with the methines at δ 4.08 and 2.19 ppm (H-1 and H-5). These correlations must arise from four and five bond couplings and were not observed in the ^{1}H nmr spectrum.

The ease of formation of anhydrodecahydrotrichodermolide (**26**) may be used to facilitate the isolation of the minor epimeric alcohol **28a**. Treatment of the epimeric mixture of alcohols **27a** and **28a** with p-toluensulphonic acid in methanol gives an easily separable mixture of anhydrodecahydrotrichodermolide (**26**) and β-decahydrotrichodermolide (**28a**).

Upon prolonged exposure of compound **26** to acidic conditions, the aromatic compound **30** is obtained. The formation of aromatic compound **30** is important since it confirms the basic carbon skeleton of trichodermolide. The structure of compound **30** was determined in the following way:

The molecular formula of **30**, $C_{23}H_{36}O_2$, was obtained from the hreims and was confirmed by cims. The ir spectrum shows the presence of ketonic (1704 cm^{-1}), and aromatic (1450, 1418 cm^{-1}) groups. The uv spectrum of compound **30** confirmed the presence of the aromatic group, with maxima at 205 (ε = 13000), 210 (15000), and 269 (400) nm. The low intensity of the aromatic C-C stretching in the ir spectrum and of the uv spectrum absorption indicates that the aromatic ring is unconjugated (66, 67).

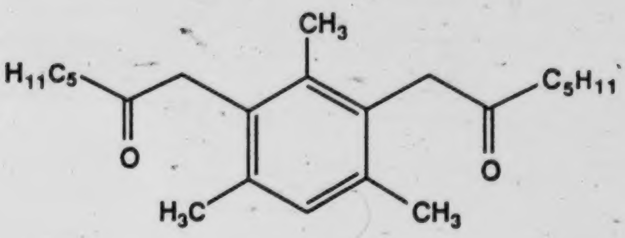
The molecular formula of **30** indicates it has six unsaturations, which are accounted for by the aromatic ring and two ketones. The ketones account for all of the oxygens in the molecular formula.

Compound **30** displays a very simple ^{1}H nmr spectrum. It shows absorptions for one aromatic hydrogen (δ 6.92 ppm), four methylene hydrogens (δ 3.76 ppm), six aromatic methyl hydrogens (δ 2.20 ppm), three aromatic methyl hydrogens (δ 2.04 ppm), all as singlets, and two hexanoyl side chains. The simplicity of the ^{1}H nmr spectrum indicates **30** possesses an element of symmetry.

The ^{13}C nmr spectrum of the aromatic compound **30** (Table 6) shows only thirteen carbons, corroborating the symmetry observed in the ^{1}H nmr spectrum. The number of carbon absorptions indicates that ten signals should correspond to twenty carbons, while three signals should correspond to three carbons. The presence of a carbon signal, corresponding to two carbons, at δ 208.6 ppm indicates the presence of two ketones. The presence of aromatic carbons (130.2, 131.0, 135.4, and 135.8 ppm) confirms the presence of an aromatic ring and the symmetry of compound **30**.

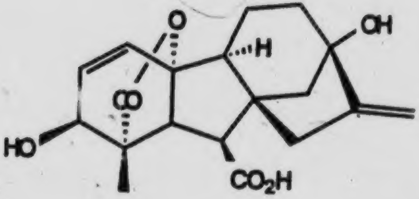
The element of symmetry present can not be a center of inversion, since the methyl at δ 2.04 and the methine at δ 6.79 ppm integrate for three and one hydrogens, respectively. Therefore the element of symmetry must be an axis of symmetry.

In order to establish the relative distribution of the aromatic substituents a series of nOe experiments was performed on **30**. The methyl at δ 2.04 ppm gives nOe enhancement to the methylenes hydrogens at δ 3.76 ppm, while the methylene hydrogens at δ 3.76 give enhancement to both aromatic methyls. These data allowed us to establish the structure of the aromatic compound as **30**.



30

The aromatization of anhydrodecahydrotrichodermolide (**26**) is not surprising since compounds containing similar functional groups are known to aromatize. For example, the acid catalyzed decarboxylation of gibberellic acid (**31**) (68) and the aromatization of 2-β-hydroxy-19-oxo-4-androstene-3,17-dione (**32**) to estrone (69) are known to occur in the presence of acid.



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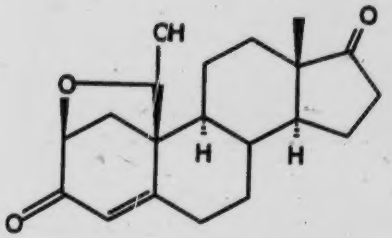

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Decahydrotrichodermolide acetates.

α-Decahydrotrichodermolide (**27a**) was acetylated by treatment with acetic anhydride to give the corresponding O-acetyl-α-decahydrotrichodermolide (**27b**). The introduction of an acetyl group in **27b** caused the expected shifts in its ^1H nmr spectrum when compared to the ^1H nmr spectrum of **27a**. Comparison of the ^1H nmr chemical shifts reveals that H-1 is, shifted downfield from δ 3.71 ppm in **27a** to δ 5.55 ppm in **27b** (1.84 ppm), which is expected for a secondary alcohol (70), and that Me-23 and Me-21 shifted upfield from δ 1.46 and 1.69 ppm in **27a** to δ 1.35 and 1.53 ppm in **27b** (0.11 and 0.16 ppm, respectively), consistent with these methyls being α to the alcohol (71) in **27a**.

Similarly, β-decahydrotrichodermolide (**28a**) was acetylated with acetic anhydride to give O-acetyl-β-decahydrotrichodermolide (**28b**). The introduction of the acetyl group caused shifts in the ^1H nmr spectrum of **28b** when compared to the ^1H nmr spectrum of **28a**: H-1 was shifted downfield from δ 3.73 ppm in **28a** to δ 5.40 ppm in **28b** (1.67 ppm) while Me-23 and Me-21 shifted upfield from δ 1.49 and 1.68 ppm in **28a** to δ 1.32 and 1.49 ppm in **28b** (0.17 and 0.19 ppm, respectively).

Table 5. ^{1}H Nmr data of trichodermolide (**19**), octahydrotrichodermolide (**25**), α-decahydrotrichodermolide (**27a**), β-decahydrotrichodermolide (**28a**), O-acetyl-α-decahydrotrichodermolide (**27b**), O-acetyl-β-decahydrotrichodermolide (**28b**), and anhydrodecahydrotrichodermolide (**26**) ($CDCl_3$, 360 MHz).

	Chemical shift in δ (ppm[a]), mult., J (Hz)						
H	19	25	27a	28a	27b	28b	26
1	-	-	3.71 d,6	3.70, br.s	5.29, br.s	5.40, br.s	4.08, br.s
5	3.34, dd,6,4	3.24, dd,7.5,4	2.71, dd,5,6.5	2.89, dd,7,3	2.73, 1 line[b]	2.92, dd,3,8.5	2.19, d,6
7	3.66, AB,19	3.28, AB,17.5	3.27, s	3.25, AB,18	3.31, s	3.29, AB,18	3.21, s
9	6.10, d,15.5	2.52, t,7	2.44, t,7.5	2.43, t,7	2.46, t,7	2.44, t,7	2.37, dt[c]
10	7.24, dd,15.5,9.5	1.60, m	1.55, m	1.56, m	1.58, m	1.58, m	1.53, m
11	6.20-5, m	1.3, m	1.3, m	1.3, m	1.3, m	1.3, m	1.04, m
12	6.20-5, m	1.3, m	1.3, m	1.3, m	1.3, m	1.3, m	1.04, m
13	1.87, d,6	0.89, t,7	0.88, t,7	0.90, t,7	0.89, t,7	0.89, t,7	0.86, t,7
14a	2.45, dd,18.5,4	2.30, dd,19,3.5	2.83, dd,18.5, 5	2.32, dd,18,3	2.71, 4 line[b]	2.43, d,18,3	4.56, d,6
14b	3.26, dd,18.5,6	3.12, dd,19,7.5	2.99, dd,18.5,7	3.19, dd,18,7	3.09, 6 line[b]	3.28, dd,18.5,8.5	
16	6.15, d,15.5	2.45, m[d]	2.50, m[d]	2.53, m[d]	2.54, m[d]	2.55, m[d]	2.04, m[d]
17	7.20, dd,15.5,9.5	1.60, m	1.55, m	1.56, m	1.58, m	1.58, m	1.43, m
18	6.20-5, m	1.3, m	1.3, m	1.3, m	1.3, m	1.3, m	1.2, m
19	6.20-5, m	1.3, m	1.3, m	1.3, m	1.3, m	1.3, m	1.2, m
20	1.89, d,6	0.91, t,7	0.87, t,7	0.89, t,7	0.90, t,7	0.89, t,7	0.86, t,6.5
21	1.78, s	1.75, s	1.68, s	1.68, s	1.53, s	1.49, s	1.68, s
22	1.24, s	1.22, s	1.06, s	1.03, s	1.10, s	1.04, s	1.17, s
23	1.45, s	1.46, s	1.46, s	1.49, s	1.35, s	1.32, s	1.43, s

[a] Relative to the residual solvent signal (7.262 ppm for chloroform). [b] The system was second order in chloroform but showed the expected coupling pattern in benzene (see Experimental). [c] The signal is not really a doublet of triplets but a complex multiplet with a predominant triplet (of J 7 Hz) shape. [d] This signal is present as a multiplet indicating that these hydrogens are diatereotopic and magnetically similar, forming an AA'BB' like system.

Table 6. ^{13}C Nmr data of trichodermolide (**19**), octahydrotrichodermolide (**25**), α-decahydrotrichodermolide (**27a**), O-acetyl-α-decahydrotrichodermolide (**27b**), anhydrodecahydrotrichodermolide (**26**), and compound **30** (CDCl$_3$, 75.5 MHz).

	Chemical shift in δ (ppm^{1,2})					
carbon	**19**^{3}	**25**^{3}	**27a**^{3}	**27b**	**26**^{3}	**30**
1	191.2	191.1	73.7	72.5	?	130.2^{a}
2	134.2	134.1	132.2	132.0	132.0	131.0^{a}
3	148.6	149.6	128.0	129.6	130.5	135.4^{a}
4	51.8	51.5	48.8	48.5	52.9	135.8^{a}
5	56.0	55.6	46.6	46.6	49.6	-
6	87.2	86.9	84.3	83.4	77.1	-
7	35.1	37.1	37.2	37.1	33.1	44.9
8	195.5^{a}	206.9^{a}	208.2^{a}	208.2^{a}	206.9	208.9
9	129.9^{b}	43.0^{b}	42.4^{b}	42.4^{b}	42.1	42.0
10	144.1^{c}	23.6^{c}	23.4	23.3^{c}	23.4	23.4
11	124.4^{d}	29.7^{d}	31.4	31.3	31.3^{a}	31.4
12	141.5^{e}	22.4	22.5	22.4	22.5	22.4
13	18.9	13.9	14.0	13.9	13.9^{b}	13.9
14	42.0	44.3	43.2	43.2	92.9	-
15	196.6^{a}	207.5^{a}	209.7^{a}	209.9^{a}	153.8	-
16	130.2^{b}	43.2^{b}	42.7^{b}	42.7^{b}	49.6	-
17	144.8^{c}	23.4^{c}	23.4	23.6^{c}	26.8	-
18	126.9^{d}	31.3^{d}	31.4	31.4	31.4^{a}	-
19	142.4^{e}	22.4^{e}	22.5	22.5	22.5	-
20	18.9	13.9	13.9	13.9	14.1^{b}	-
21	11.6	11.5	20.4	20.8^{d}	20.2	20.6
22	16.2	16.1^{f}	16.2^{c}	15.7	17.2^{c}	16.7
23	16.2	16.3^{f}	15.7^{c}	15.6	15.6^{c}	-
24	176.1	176.0	177.7	177.0	178.5	-
CO	-	-	-	169.6	-	-
Me	-	-	-	19.7^{d}	-	-

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[1] The assignments of the signals with the same superscript in the same column are interchangeable. [2] Relative to the carbon of the solvent (77.0 for chloroform). [3] APT obtained

Table 7. ^{1}H Nmr nOe experiments with trichodermolide (**19**), octahydrotrichodermolide (**25**), α-decahydrotrichodermolide (**27a**), O-acetyl-α-decahydrotrichodermolide (**27b**), β-decahydrotrichodermolide (**28**), and anhydrodecahydrotrichodermolide (**26**) (CDCl$_3$, 360 MHz).

Signal saturated	Signal enhanced (% nOe[a])
19	
1.24 (H-22)	3.66 (H-7) (4) 3.34 (H-5) (10) 3.26 (H-14b) (3)
1.45 (H-23)	2.45 (H-14a) (6) 3.34 (H-5) (9)
1.78 (H-21)	3.66 (H-7) (7)
2.45 (H-14a)	3.34 (H-5) (8) 3.26 (H-14b) (20) 6.15 (H-16) (3) 7.20 (H-17) (3.5)
3.34 (H-5)	1.24 (H-22) (2) 1.45 (H-23) (3) 2.45 (H-14a) (10)
3.66 (H-7)	1.78 (H-21) (11) 1.24 (H-22) (3) 6.10 (H-9) (15) 7.24 (H-10) (19)
3.26 (H-14b)	2.45 (H-14a) (27) 6.10 (H-16) (6) 7.20 (H-17) (7)
25	
2.71 (H-5)	1.46 (H-23) (5)
1.22 (H-22)	3.24 (H-5) (13) 3.48 (H-7) (8)
1.46 (H-23)	2.30 (H-14a) (8) 3.24 (H-5) (10)
1.75 (H-21)	3.28 (H-7) (6)
3.24 (H-5)	1.22 (H-22) (1) 1.46 (H-23) (1) 2.30 (H-14a) (3.5)
27a	
1.06 (H-22)	2.71 (H-5) (2.3) 3.27 (H-7) (3.8)
1.46 (H-23)	2.71 (H-5) (3.1) 2.83 (H-14a) (1) 3.71 (H-1) (2.7)
1.67 (H-21)	3.27 (H-7) (1.7) 3.71 (H-1) (1.5)
3.27 (H-7)	2.44 (H-9) (5) 1.69 (H-21) (7) 1.06 (H-22) (4)
3.71 (H-1)	2.22 (OH-1) (6) 1.69 (H-21) (4) 1.46 (H-23) (4) 1.06 (H-22) (4)

Table 7 continued.

Signal saturated	Signal enhanced (% nOe[a])
27b	
1.10 (H-22)	3.31 (H-7) (3) 2.73 (H-5) (3)
1.35 (H-23)	5.29 (H-1) (2.5) 2.73 (H-5) (1)
1.53 (H-21)	5.29 (H-1) (2.5) 3.31 (H-7) (2)
3.31 (H-7)	1.53 (H-21) (7) 2.46 (H-9) (4) 1.10 (H-22) (1)
28a	
1.03 (H-22)	3.25 (H-7) (1) 2.89 (H-5) (5)
1.49 (H-23)	3.72 (H-1) (3) 2.89 (H-5) (3)
1.69 (H-21)	3.72 (H-1) (2) 3.25 (H-7) (0.8)
26	
1.17 (H-22)	2.19 (H-5) (3.3) 3.21 (H-7) (2) 4.56 (H-14) (1)
1.43 (H-23)	2.04 (H-16 (1) 2.19 (H-5) (2.5) 4.08 (H-1) (3.3)
1.68 (H-21)	3.21 (H-7) (2) 4.08 (H-1) (3.5)
2.19 (H-5)	1.17 (H-22) (6) 1.43 (H-23) (8) 4.56 (H-14) (10)
3.21 (H-7)	1.17 (H-22) (4) 1.68 (H-21) (5.4) 2.37 (H-9) (1)
4.08 (H-1)	1.43 (H-23) (7) 1.68 (H-21) (5.4)
4.56 (H-14)	2.04 (H-16) (5) 2.19 (H-5) (11) 2.37 (H-9) (1)

[a] Ratio of the enhanced area over the irradiated signal's area in the difference spectra.

Absolute stereochemistry of trichodermolide (19).

The determination of the absolute stereochemistry of trichodermolide (**19**) by analysis of its CD spectrum is complicated since the CD spectrum of trichodermolide (Figure 10) shows a series of Cotton effects which do not resemble a split Cotton effect. There are three chromophores in trichodermolide: the two sorbyl chains and the α,β-unsaturated ketone*. This situation is more complicated than the two chromophore situation observed in trichodimerol and results in a complex CD spectrum.

Since it is very difficult to predict how the dipole moment change vectors of three chromophores are going to interact, we were unable to reach an unambiguous conclusion regarding the absolute stereochemistry of trichodermolide on the basis of its CD spectrum. We therefore examined the CD spectrum (Figure 11) of one of its derivatives: octahydrotrichodermolide (**25**). This allowed the assignment of the absolute stereochemistry of **25**, and therefore of trichodermolide. The CD spectrum of octahydrotrichodermolide displays a split Cotton effect with extrema of opposite sign at 270 and 222 nm (*A* = +28, positive chirality). The reason for the split Cotton effect was not apparent at first. However, upon careful examination of the uv spectrum of octahydrotrichodermolide we concluded that the α,β-unsaturated ketone in octahydrotrichodermolide is an inherently disymmetric chromophore. This arises by homoconjugation of the lactone carbonyl with the double bond of the α,β-unsaturated ketone. Examination of molecular models reveals that the π bond of the lactone carbonyl of octahydrotrichodimerol is in a favoured position to homoconjugate with the double bond of the α,β-unsaturated ketone.

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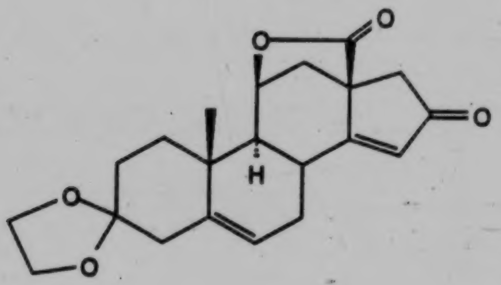
* Which in itself is more complicated as explained latter (vide infra).

The uv spectrum of octahydrotrichodermolide shows maxima and extinction coefficients that do not agree with the predicted values for a trisubstituted α,β-unsaturated ketone (72). The predicted maxima for the charge transfer band in a disubstituted cyclohexenone is 237 nm with an absorptivity of about 10000. The n-π^* transition is almost independent of substituent effects and absorbs around 325 nm with "ill-defined" fine structure and an absorptivity of about 100 (72). In octahydrotrichodermolide the charge transfer band is found at longer wavelengths (272 nm) and has a weaker absorptivity (5600), while the n-π^* band has an increased one (1200). These shifts and intensity changes confirm the suspected homoconjugative effect (73, 74).

Homoconjugation of lactonic carbonyls with enones has been documented. For example compound **33** shows a similar homoconjugative effect, with maxima at 222 (ε = 10000) and 241 (12000) nm, while the predicted maximum is at 229 nm (75). Furthermore, Ellestad and co-workers (76) reported the uv and CD spectra of compound **34**. The uv spectrum of compound **34** shows maxima at longer wavelengths (260-265 nm) than other abietane derivatives with similar structure and a C-10 methyl (77). The CD spectrum of compound **34** shows a split Cotton effect, with extrema at 262 and 230 nm (A = +74.4, positive chirality). The absolute stereochemistry of **34** is known by chemical correlation with compound **35** (76). The absolute stereochemistry of compound **35** was determined by use of the dibenzoate chirality rule (78) on compound **36**, the 6,7-di-*p*-methoxybenzoate derivative of **35**. The CD spectrum of compound **36** shows maxima at 280 and 253 nm (*A* = -24.2, negative chirality).

Interestingly, compound **34** possesses virtually the same chromophore as octahydrotrichodermolide (**25**). Compound **34** and octahydrotrichodermolide show the same chirality in their CD spectra. Since the absolute stereochemistry of compound **34** is known, we assign the absolute stereochemistry of trichodermolide as shown in **19**.



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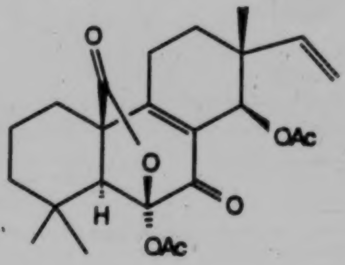
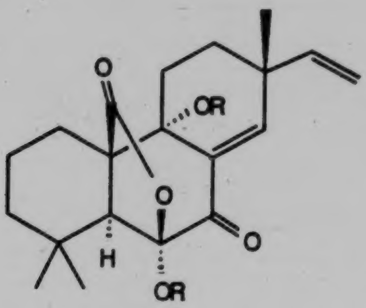


34



35 R= H
36 R = -COPh

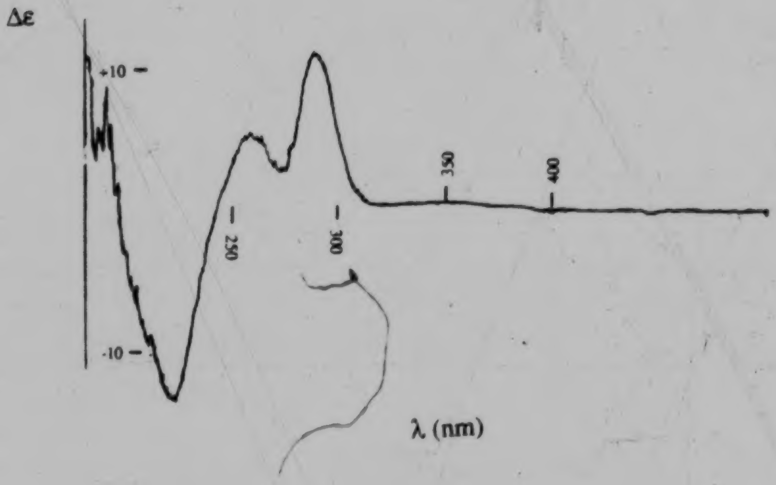


Figure 10. CD spectrum of trichodermolide (19) (MeOH).

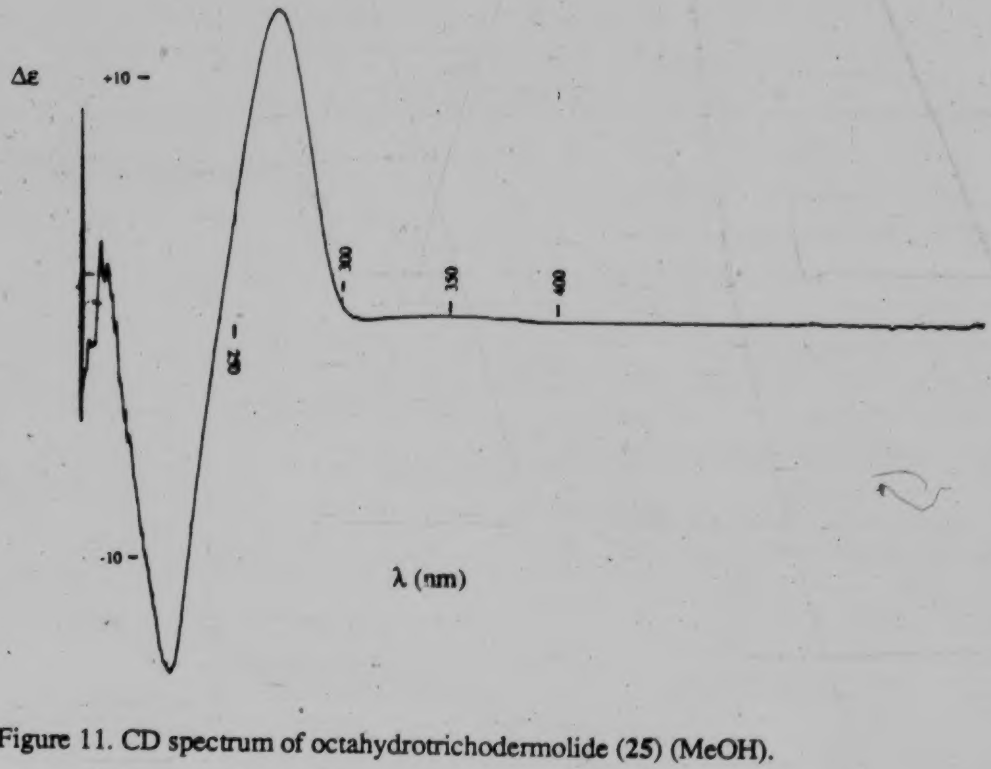


Figure 11. CD spectrum of octahydrotrichodermolide (25) (MeOH).

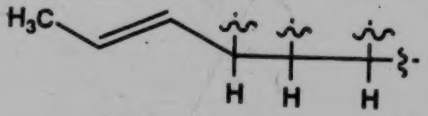
Sorbiquinol (37)

Another polyketide isolated from the mycelium of *T. longibrachiatum* has been named sorbiquinol. It is an optically active ($[\alpha]_D$ +234°(*c* 0.36, MeOH)) pale yellow substance. Its uv spectrum (Table 11) shows maxima at 292, 359, and 374 nm, which shift upon addition of base (252, 283, and 352 nm), thus indicating the presence of acid chromophore(s). The molecular formula, $C_{28}H_{32}O_7$, was obtained from the hreims and the molecular ion was confirmed by the cims. Its mass spectrum has a fragmentation pattern similar to that of sorbicillin. The mass spectrum of sorbiquinol shows fragment ions at m/z 452, corresponding to the loss of carbon monoxide, at m/z 95 corresponding to a sorbyl chain, and the base peak, at m/z 165, corresponding to the stabilized acylium ion **2**.

The ir spectrum shows absorption for hydroxyl (3450 cm^{-1}), ketone (1730 cm^{-1}), strongly chelated carbonyl (1627 cm^{-1}), and double bond (1606 cm^{-1}) groups.

The ^1H nmr spectrum of sorbiquinol (Table 8) shows hydrogen signals for two hydroxyls, two methyls as singlets, two aromatic methyls, an aromatic methine, a chelated enol, a chelated phenol, a sorbyl chain, a three hydrogen spin system {δ 3.26 (dd, 6.5, 10 Hz, 1), 3.32 (d, 1.5 Hz, 1H), 4.28 (dd, 6.5, 1.5 Hz, 1H) ppm}, and E-1-propenyl {δ 1.60 (dd, 6.5, 1.5 Hz, 3H), 5.02 (ddq, 15, 10, 1.5 Hz, 1H), 5.45 (dq, 15, 6.5 Hz, 1H) groups.

Decoupling experiments allow us to assign part of the ^1H nmr spectrum and to formulate fragment **38**. The methyl at δ 1.60 ppm is coupled to the hydrogens at δ 5.45 ppm (6.5 Hz), and δ 5.02 (1.5 Hz). The hydrogen at δ 5.02 ppm which shows *trans* coupling (15 Hz) to the hydrogen at δ 5.45 ppm is also coupled to the hydrogen at δ 3.26 ppm (10 Hz), which in turn is coupled to the hydrogen at δ 4.28 ppm (6.5 Hz). The hydrogen at δ 4.28 is also coupled to the hydrogen at δ 3.32 ppm (1.5 Hz). The remaining alkenic hydrogens signals have the coupling pattern of a sorbyl chain.



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The ^{13}C nmr spectrum (Table 9) shows 28 carbons. Six signals are assigned to methyl, three to aliphatic methines, six to sp^2 methines, one to a quaternary carbon (δ 63.2 ppm), one to an oxygen bearing tertiary carbon (δ 75.7 ppm), six to oxygen bearing sp^2 carbons, and four to non-hydrogen bearing sp^2 carbons.

In order to gather further insight into the structure of sorbiquinol, a series of nOe experiments was performed (Table 10) using acetone* as a solvent. The aromatic hydrogen at δ 7.69 ppm gives a strong (26%) nOe enhancement of the hydrogen at δ 4.48 ppm and an enhancement of the methyl at δ 2.24 ppm.

The spectra of sorbiquinol and sorbicillin have similarities. In the ^{1}H nmr spectra of each compound the chemical shift of the aromatic methyls, the aromatic methine, and chelated phenol are similar. The mass spectrum of sorbiquinol and that of sorbicillin each show a base peak at m/z 165 while an nOe experiment on each compound indicates that the aromatic methine is adjacent to a methyl group. These data suggest that sorbiquinol, like sorbicillin, contains a pentasubstituted aromatic ring. This information, together with the observation that there is a strong nOe between the aromatic methine and the hydrogen at δ 4.48 ppm allow the formulation of fragment **39**.

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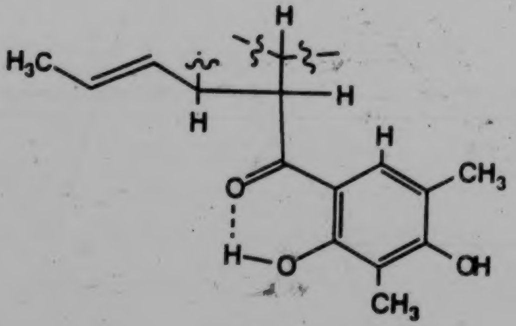
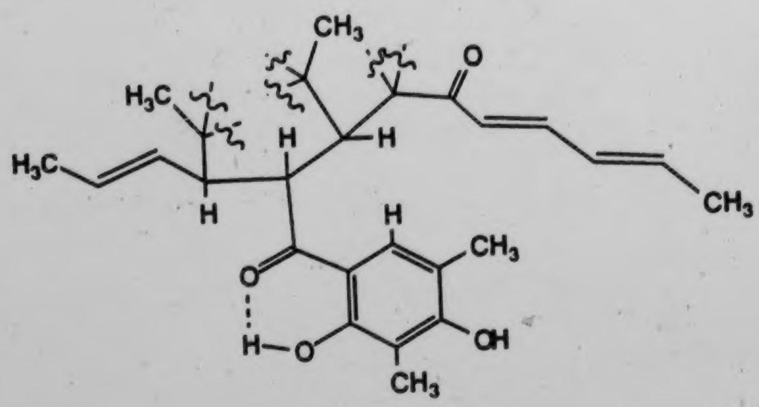
* The nOe experiments were done in acetone-d$_6$ in order to increase the size of the nOe enhancements observed. The chemical shifts of the signals are those in acetone-d$_6$.



39

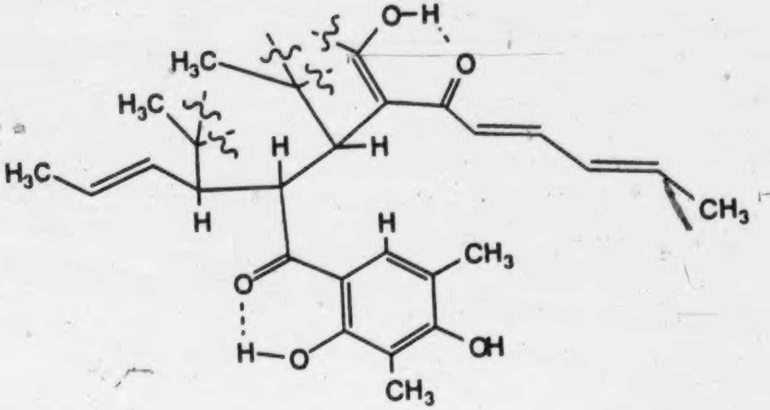
At this stage further information on the relative distribution of the groups present in sorbiquinol was provided by the nOe experiments on sorbiquinol. The methine at δ 3.50 ppm shows nOe with the methyl at δ 1.20 ppm, indicating that the methyl is vicinal to this methine. Similarly, the methyl at δ 1.07 ppm shows an nOe enhancement to the methine at δ 3.19 ppm indicating that these groups also are vicinal. In addition, the methine at δ 3.50 ppm shows a strong nOe enhancement (25%) with the sorbyl chain hydrogen at δ 5.82 ppm. These observations allow us to expand fragment **39** to fragment **40** and establish the relative location of the sorbyl chain.



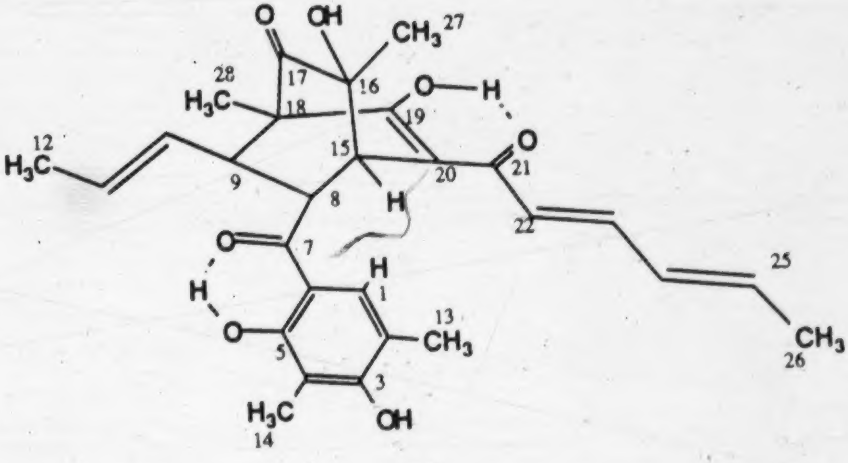
40

Four exchangeable hydrogens can be deduced from the ^{13}C nmr and mass spectral data of sorbiquinol. Two of the hydrogens are accounted for by the chelated enol and the chelated phenol while one is accounted for by the phenol. From this information together with the ^{13}C nmr data we can deduce that the remaining exchangeable hydrogen may be attributed to a tertiary alcohol.

There are seven oxygen bearing carbons in the ^{13}C nmr spectrum. Fragment **40** accounts for four of these. If the remaining three oxygen bearing carbons are assigned as an enol, a tertiary alcohol, and a ketone, then all the oxygen bearing carbons observed in the ^{13}C nmr spectrum are accounted for. The chemical shift of the ketonic carbon (δ 211.6 ppm), indicates that this carbonyl is not conjugated. This allows the construction of fragment **41** and leads to the derivation of structure **42** for sorbiquinol. This structure accommodates all of the spectral data obtained for sorbiquinol.


O-H
O
H₃C
H₃C
H
H
H₃C
H
CH₃
H
CH₃
O
H-O
OH
CH₃

41


O
OH
CH₃ 27
28
H₃C
17
16
18
O-H
19
15
O
12
H₃C
9
20
21
8
H
22
7
O
H
1
25
H
13
O
5
CH₃
CH₃
3
26
H₃C
14
OH

42

Relative Stereochemistry of Sorbiquinol (37).

Having established the constitution of sorbiquinol as **42**, we turned our attention to the determination of its relative stereochemistry. The relative stereochemistry of carbons C-8, C-9, and C-16 must be assigned. The size of the coupling constant between H-9 and H-8 (6.5 Hz) did not allow an assignment of the relative stereochemistry at C-8 and C-9 since its value does not allow distinction between a *trans* and a *cis* coupling (J_{trans} 2.8-6.6 Hz, J_{cis} 6.7-9.2 Hz, (79)).

However, the results from the nOe experiments proved very useful. The hydrogen at C-8 (δ 4.48 ppm) shows a strong nOe enhancement with H-10 (δ 5.16 ppm), thus establishing that the 1-propenyl group is *cis* to H-8. The absence of enhancement between Me-27 (δ 1.20 ppm) and H-8 (δ 4.48 ppm) suggests that Me-27 and H-8 are on opposite sides of the molecule, but this was not considered sufficient evidence to establish the relative stereochemistry at C-16. The results from the nOe experiments are summarized in Table 10 and may be translated into a network of nOe enhancements, as shown in Figure 12.

A ^{1}H nmr pyridine solvent shift study of sorbiquinol enabled us to settle this point. Upon addition of five drops of pyridine-d_5 to the $CDCl_3$ solution of sorbiquinol, H-8 and H-10 were shifted downfield by 0.27 and 0.17 ppm relative to their chemical shifts in $CDCl_3$, while addition of a total of ten drops shifted H-8 and H-10 by 0.37 and 0.24 ppm, respectively. The magnitude of the solvent shifts indicates that the C-16 hydroxyl is *cis* to H-8 and C-10 (36), and thus establishes the relative stereochemistry of C-16 to be as shown in structure **37**.

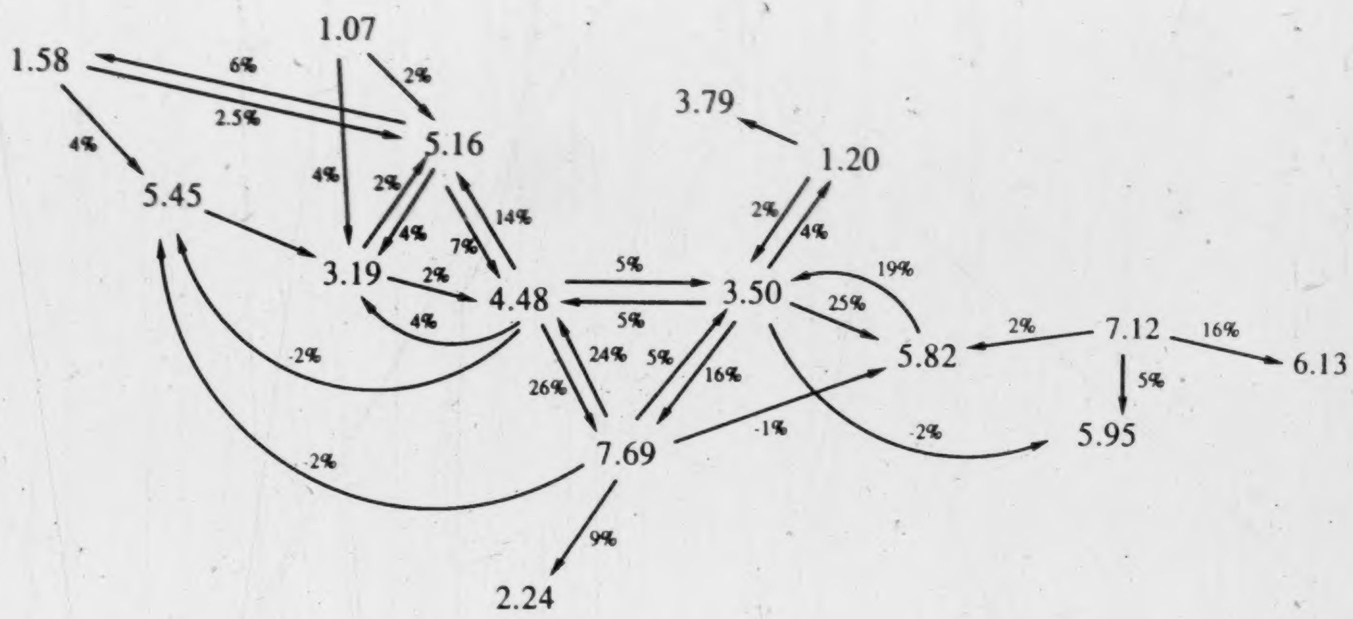
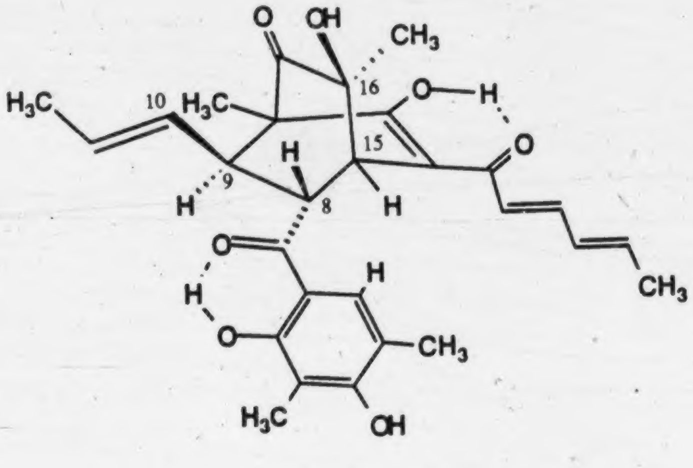

1.58
1.07
5.16
5.45
3.19
4.48
3.79
1.20
3.50
5.82
7.12
6.13
5.95
7.69
2.24
6%
2.5%
4%
2%
4%
2%
4%
7%
14%
2%
4%
-2%
-2%
5%
5%
24%
26%
5%
16%
2%
4%
19%
25%
-1%
-2%
2%
16%
5%
9%

Figure 12. NOe enhancements observed in sorbiquinol (37) (CDCl3, 360 MHz).

The chemical shift of Me-27 supports the assigned relative stereochemistry at C-16. A methyl on an oxygen bearing carbon resonates at about δ 1.45 ppm (80) as is observed in the ^1H nmr spectra of trichodimerol and trichodermolide. The high field chemical shift of Me-27 in sorbiquinol (δ 1.20 ppm) is explained, however, if Me-27 lies in the shielding region of the enol-sorbyl system. Such is the case for the proposed structure of sorbiquinol and the observed shielding lends support to the assigned stereochemistry at C-16.

The structure of sorbiquinol is comprised of two sorbicillin units. A retro Diels Alder reaction of sorbiquinol would be expected to give a sorbicillin unit and an oxidized sorbicillin unit as shown in Scheme 1. This suggests a possible biogenesis of sorbiquinol as is discussed later.



37

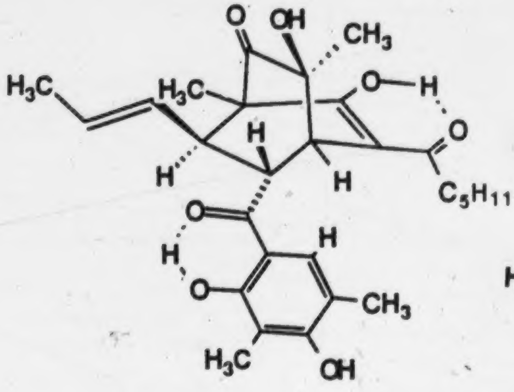
Hydrogenation of Sorbiquinol (37).

Tetrahydrosorbiquinol (43).

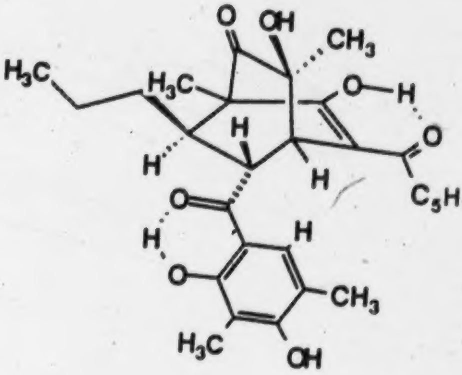
Tetrahydrosorbiquinol was prepared by hydrogenation of sorbiquinol over palladium on carbon in ethyl acetate for one hour. The molecular formula, $C_{28}H_{36}O_7$, was obtained from the hreims, and the molecular ion was confirmed by cims. The mass spectrum, as anticipated, also shows a hexanoyl chain, at m/z 99, and the base peak, at m/z 165, corresponding to the stabilized acylium ion **2**, as is observed in the mass spectra of sorbiquinol. The ir spectrum of tetrahydrosorbiquinol shows the presence of a hydroxyl (3450 cm^{-1}), a ketone (1730 cm^{-1}), and a strongly chelated carbonyl (1625 cm^{-1}). The uv spectrum is similar to that of tetrahydrosorbicillin (**3**) (Table 11), indicating that they have the same pentasusbtituted aromatic chromophore.

The ^1H nmr spectrum of tetrahydrosorbiquinol (Table 8) shows the expected changes when compared with the ^1H nmr spectrum of sorbiquinol. Signals attributed to a sorbyl side chain are absent but signals corresponding to a hexanoyl chain (Table 8) are observed. The enolized β-diketone absorption of **43** shifted with respect to sorbiquinol to δ 14.37 ppm, corroborating the hydrogenation of the sorbyl chain. The ^1H nmr spectrum of tetrahydrosorbiquinol (**43**) is assigned with the aid of nOe experiments (Table 10) and, except for the changes noted, it is virtually unchanged from that of sorbiquinol. Under these conditions, the double bond of the propenyl group was not hydrogenated (see Table 8).

The ^{13}C nmr spectrum of **43** (Table 9) shows the expected changes when compared to the ^{13}C nmr spectrum of sorbiquinol (**37**). The sorbyl group resonances in sorbiquinol are replaced by the resonances of a hexanoyl chain, and one of the oxygen bearing carbons of the enolized β-diketone is shifted downfield to δ 180.4 ppm in tetrahydrosorbiquinol.



43



44

Hexahydrosorbiquinol (44).

When the hydrogenation of sorbiquinol is allowed to proceed for two days, a hexahydro derivative (**44**) is obtained. The molecular formula, $C_{28}H_{38}O_7$, was obtained from the hreims and confirmed by cims. The base peak in the mass spectrum of **44** is the stabilized acylium ion **2**, at m/z 165. The ir spectrum of hexahydrosorbiquinol shows absorption for hydroxyl (3480 cm^{-1}), ketone (1732 cm^{-1}), and strongly chelated carbonyl

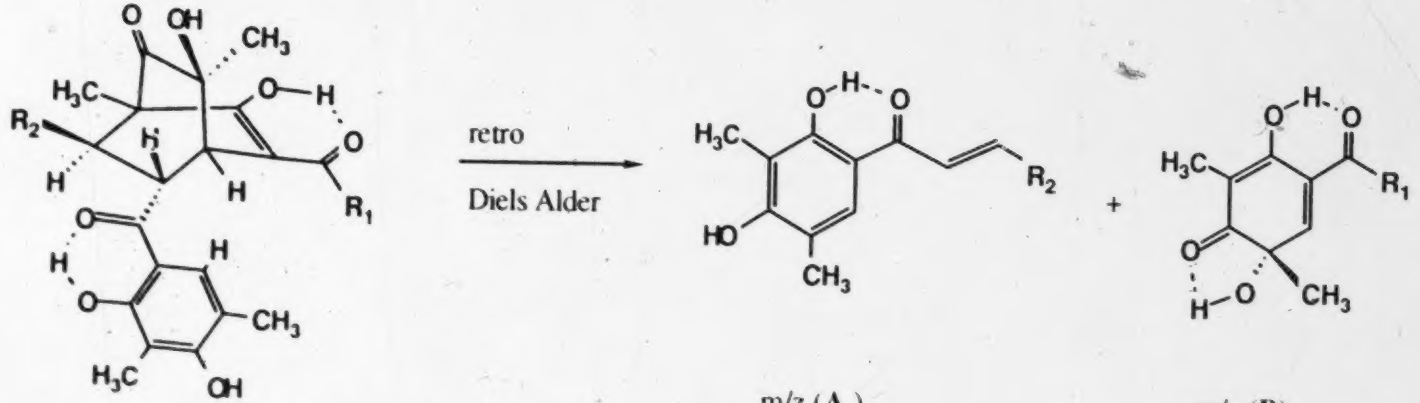
(1627 cm^{-1}) groups, and the uv spectrum is virtually identical to that of tetrahydrosorbiquinol.

The ^1H nmr spectrum of hexahydrosorbiquinol (**44**) is very similar to the ^1H nmr spectrum of tetrahydrosorbiquinol. The ^1H nmr signal of Me-28 in hexahydrosorbiquinol (**44**) (δ 1.29 ppm) is shifted downfield (0.13 ppm) when compared to the ^1H nmr signal of Me-28 in tetrahydrosorbiquinol (**43**) (δ 1.16 ppm), indicating that this methyl lies in the shielding region of the double bond. The propenyl signals present in ^1H nmr spectrum of tetrahydrosorbiquinol are not present in the ^1H nmr spectrum of **44**, and as expected, the H-9 and H-8 signals are shifted upfield to δ 2.72 ppm (0.52 ppm) and δ 4.18 ppm (0.09 ppm) in hexahydrosorbiquinol. This is consistent with the saturation of the propenyl chain in hexahydrosorbiquinol. This change was also reflected in the ^{13}C nmr spectrum of hexahydrosorbiquinol which, except for the carbons assigned to the aromatic ring, does not show any signals for double bond carbons (Table 9).

Sorbiquinol and its two hydrogenated derivatives show fragment ions in their mass spectra which may arise form a retro Diels Alder reaction. The chemical ionization mass spectrum of sorbiquinol shows two fragment ions at m/z 233, the base peak, and at m/z 249. These ions may arise from a retro Diels Alder reaction of sorbiquinol, as shown in Scheme 1. The corresponding fragments are also observed in the chemical ionization mass spectra of tetrahydrosorbiquinol and hexahydrosorbiquinol. The chemical ionization spectrum of tetrahydrosorbiquinol shows the fragment ions at m/z 233, the base peak, and at m/z 253, corresponding to the protonated retro Diels Alder fragments. The chemical ionization mass spectrum of hexahydrosorbiquinol shows these fragment ions at m/z 235 and 253. In hexahydrosorbiquinol the base peak is the protonated molecular ion. This provides further support for the assigned structures.

Absolute Stereochemistry of Sorbiquinol (37).

Having determined the relative stereochemistry, we focussed our attention on the absolute stereochemistry of sorbiquinol. The CD spectra of sorbiquinol, tetrahydrosorbiquinol, and hexahydrosorbiquinol display a split Cotton effect with positive chirality. However, the direction of the electric transition dipole moment vector of the aromatic chromophore cannot be readily predicted. Therefore, the assignment of the absolute stereochemistry of sorbiquinol cannot be made unambiguously. However, the similarity between the oxygenated sorbicillin subunit present in both sorbiquinol and in trichodimerol suggests that the absolute stereochemistry of the tertiary alcohol may be the same in sorbiquinol and in trichodimerol. On the basis of this similarity, we assign the absolute stereochemistry of sorbiquinol as shown in **37**.



	m/z (A)	m/z (B)
37 $R_2 = C_3H_5$, $R_1 = C_5H_7$, 480	232	248
43 $R_2 = C_3H_5$, $R_1 = C_5H_{11}$, 484	232	252
44 $R_2 = C_3H_7$, $R_1 = C_5H_{11}$, 486	234	252 (253)

Scheme 1

Retro Diels Alder fragments of sorbiquinol (**37**), tetrahydrosorbiquinol (**43**), and hexahydrosorbiquinol (**44**).

Table 8. ^{1}H Nmr data of sorbiquinol (**37**), tetrahydrosorbiquinol (**43**), hexahydrosorbiquinol (**44**), sorbicillin (**1**), and tetrahydrosorbicillin (**3**) ($CDCl_3$, 360 MHz).

	Chemical shift in δ (ppm[a]), mult., *J* (Hz)				
H	**37**	**43**	**44**	**1**	**3**
1	7.59, s	7.60, s	7.64, s	7.48, s	7.39, s
3 (OH)	-	-	5.36, s	5.26, s	5.32, s
5 (OH)	12.58, s	12.79, s	12.84, s	13.58, s	13.04, s
8	4.28, dd,6.5,1.5	4.27, dd,7,1.5	4.18, dd,7,1.5	6.98, d,15	2.89, t,7
9	3.26, dd,10,6.5	3.245[b], dd,6.5,10	2.72, m	7.48, dd,10,15	1.72, m
10	5.02, ddq,15,10,1.5	4.99, ddq,15,10,1.5	1.1, m	6.38, dd,10,12	1.3, m
11	5.45, dq,16,6.5	5.44, dq,15,7	1.1, m	6.32, dq,6,12	1.3, m
12	1.60, dd,6.5,1.5	1.60, dd,1.5,7	0.79, m	1.92, d,6	0.91, t,7
13	2.26, s	2.26, s	2.27, s	2.24, s	2.21, s
14	2.12, s	2.13, s	2.14, s	2.16, s	2.13, s
15	3.32, d,1.5	3.247[b], d,1.5	3.22, d,1.5		
19 (OH)	13.98, s	14.37, s	14.38, s		
22	5.53, d,15	1.96, t,8	1.94, t,7		
23	7.17, dd,15,10.5	1.45, m	1.47, m		
24	5.93, ddq,15,10,1.5	1.0, m	1.1, m		
25	6.08, dq,15,7	1.0, m	1.1, m		
26	1.82, dd,7,1.5	0.78, t,7.5	0.79, m		
27	1.20, s	1.23, s	1.21, s		
28	1.16, s	1.16, s	1.29, s		
16 (OH)	-	2.87, s	2.81, s		

[a] Relative to the residual solvent signal (7.262 for chloroform). [b] The chemical shift and multiplicity of these signals was obtained from the nOe difference spectra since the signals overlap in the regular ^{1}H nmr spectrum.

Table 9. ^{13}C Nmr data of sorbiquinol (37), tetrahydrosorbiquinol (43), hexahydrosorbiquinol (44), sorbicillin (1), and tetrahydrosorbicillin (3) (CDCl$_3$, 75.5 MHz).

	Chemical shift in δ (ppm[1,2])				
Carbon	**37**[3]	**43**[3,4]	**44**[3,4]	**1**[3]	**3**
1	129.0	129.0	128.7	128.2	129.2
2	115.0[a]	115.0[a]	115.2[a]	113.9	114.6[a]
3	159.0	159.0	159.0	158.2	158.8
4	110.7	110.6	110.8[a]	109.9	110.4
5	161.9	162.0	162.3	162.0	161.4
6	112.0[a]	111.9[a]	111.3[a]	113.0	112.9[a]
7	202.1	202.2	202.9	192.0	205.5
8	46.7[b]	47.0[b]	46.4[b]	121.3	37.9
9	46.5[b]	46.5[b]	42.7[b]	144.0	24.8
10	128.6	127.2	33.3	130.0	31.6
11	130.6	130.5	20.7	140.6	22.5
12	17.8	17.8	14.0	18.3	13.9
13	16.0	15.7	16.0	15.1	15.6
14	7.5	7.5	7.5	7.0	7.5
15	47.0[b]	47.0[b]	46.9[b]		
16	75.7	75.4	75.4		
17	211.6	211.4	211.6		
18	63.2	62.6	63.1		
19	168.8	180.7	180.4		
20	106.8	106.3	106.4		
21	198.0	196.9	197.5		
22	117.2	31.7	32.7		
23	142.4	26.1	26.1		
24	130.8	31.5	31.6		
25	139.4	22.1	22.1		
26	18.9	13.9	13.8		
27	24.3	24.3	24.4		
28	10.0	9.7	9.7		

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[1] Signals with the same superscript in the same column are interchangeable.

[2] Relative to the carbon of the solvent (77.0 for chloroform). [3] APT obtained.

[4] Also obtained [1-^{13}C]acetate incorporated sorbiquinol.

Table 10. ^{1}H Nmr nOe experiments with sorbiquinol (**37**), tetrahydrosorbiquinol (**43**), and hexahydrosorbiquinol (**44**) (360 MHz).

Signal saturated	Signal enhanced (%nOe^a)	Signal saturated	Signal enhanced (%nOe^a)
37 (acetone-d_6)			
1.07 (H-28)	3.19 (H-9) (4) 5.16 (H-10 (2)	7.12 (H-23)	5.82 (H-22) (2) 5.95 (H-24) (5) 6.13 (H-25 (16)
1.20 (H-27)	3.50 (H-15) (2) 3.79 (OH-16) (0.7)	7.69 (H-1)	2.24 (H-13) (9) 4.48 (H-8) (24) 3.50 (H-15) (5) 5.82 (H-22) (-1) 5.16 (H-10) (-2) 5.45 (H-11) (-2)
1.58 (H-12)	5.16 (H-10) (2.5) 5.45 (H-11) (4)		
3.19 (H-9)	4.48 (H-8) (2) 5.16 (H-10) (2)		
3.50 (H-15)	1.20 (H-27) (4) 4.48 (H-8) (5) 5.82 (H-22) (25) 5.95 (H-24) (-2) 7.69 (H-1) (16)	**43** (CDCl$_3$) 1.16 (H-28)	3.245 (H-9) (2.3) 4.99 (H-10) (1.1) 5.44 (H-11) (0.4)
4.48 (H-8)	3.19 (H-9) (4) 3.50 (H-15) (5) 5.16 (H-10) (14) 5.45 (H-11) (-2) 7.69 (H-1) (26)	1.23 (H-27) 1.96 (H-22) 2.26 (H-13)	3.247 (H-15) (2) 3.247 (H-15 (7) 7.60 (H-1) (2)
5.16 (H-10)	4.48 (H-8) (7) 3.19 (H-9) (4) 1.58 (H-12) (6)	4.27 (H-8)	3.25 (H-15, H-9) (7) 4.99 (H-10) (13) 7.60 (H-1) (23)
5.45 (H-11)	3.19 (H-9) (9)	5.44 (H-11)	3.245 (H-9) (10) 4.99 (H-10) (2)
5.82 (H-22)	3.50 (H-15) (19)		

Table 10 continued.

Signal saturated	Signal enhanced (%nOe[a])
7.60 (H-1)	2.26 (H-13) (7) 3.247 (H-15) (6) 4.27 (H-8) (22) 4.99 (H-10) (-2.4)
44 ($CDCl_3$)	
1.21 (H-27)	1.47 (H-23) (3) 2.81 (OH-16) (1) 3.22 (H-15) (15)
1.29 (H-28)	2.72 (H-9) (2)
1.94 (H-22)	1.47 (H-23) (10) 3.22 (H-15) (7)
3.22 (H-15)	1.21 (H-27) (3) 4.18 (H-8) (4) 7.64 (H-1) (5)
7.64 (H-1)	3.22 (H-15) (6) 4.18 (H-8) (19)

[a] Ratio of the enhanced area over the irradiated signal's area in the difference spectra.

Table 11. Uv data of sorbiquinol (**37**), tetrahydrosorbiquinol (**43**), hexahydrosorbiquinol (**44**), sorbicillin (**1**), and tetrahydrosorbicillin (**3**) (MeOH).

Compound	λ_{max} nm (ε)[a]	λ_{max} nm (ε) in base[b]
37	292 (23000), 359 (22500), 374 (19500)	252 (18200), 283 (18300), 352 (27000)
43	217 (13800), 232 sh, 290 (19000)	205 (113000), 258 (12400) 309 sh, 344 (29000)
44	215 (15900), 230 sh, 289 (18300), 330 sh	202 (45000), 257 (6900), 315 sh, 344 (23000)
1	318 (27000)	405 (29000)
3	214 (14700), 229 sh, 284 (10400), 327 (4500)	204 (43000), 253 (5400), 346 (21000)

[a] In neutral solution. [b] Upon addition of a drop of 5% sodium hydroxide. In all cases acid regenerated the original spectrum.

Bisvertinol (45)

The compound responsible for the strong yellow coloration of the broth was isolated from the still culture broth of *T. longibrachiatum*. Compound **45** is a bright yellow, optically active solid (mp 151.5-152.5°C). Its ir spectrum shows absorption for hydroxyl (3400 cm^{-1}) and strongly chelated carbonyl (1616 cm^{-1}) groups while its uv spectrum shows maxima at 400 ($\varepsilon = 24000$), 300 (15000), 274 (15400), and 227 (9900) nm. The uv spectrum shifts to longer wavelength (426, 362, 298, 273, 234, 204 nm) upon addition of base, indicating that the chromophore in compound **45** has acidic characteristics.

The ^1H nmr spectrum (chloroform) of compound **45** shows signals corresponding to methyls (δ 1.30, 1.38, 1.50, and 1.58 ppm), two sorbyl chains {δ 1.92 (d, 7 Hz, 6H), 6.4 (m, 7H), 7.34 (dd, 15, 11 Hz, 2H)}, strongly chelated enols (δ 16.2 and 16.7 pm), a methylene {δ 2.62 (d, 14 Hz, 1H) and 2.75 (d, 14 Hz, 1H) ppm}, a hydroxyl (δ 3.98 ppm), and a methine (δ 3.68 ppm).

The ^{13}C nmr spectrum of compound **45** shows 28 carbons: signals corresponding to six methyls, a methylene carbon (δ 36.5 ppm), a methine carbon (δ 54.9 ppm), a quaternary carbon (δ 60.3ppm), tertiary carbons bearing oxygen (δ 74.2 and 80.4 ppm), an hemiacetal carbon (δ 106.0 ppm), electron rich sp^2 carbons (δ 102.5, 107.2, and 110.6), eight sp^2 carbons as doublets, and several carbonyl like carbons (δ 168.4, 170.0, 179.7, 193.3, and 194 ppm).

The ^{13}C nmr data is consistent with a molecular formula with at least 28 carbons, 29 hydrogens, and 8 oxygens for compound **45**. Four exchangeable hydrogens are clearly observed in the ^{1}H nmr spectrum of compound **45** (the two chelated enols and the two hydroxyl hydrogens). The multiplet at δ 6.4 ppm integrates for seven hydrogens, six of which correspond to hydrogens of the sorbyl chains. Since the 29 hydrogens obtained from the ^{13}C nmr data have been accounted for, the remaining hydrogen at δ 6.4 ppm must be exchangeable, thus there are five exchangeable hydrogens in compound **45**. Adding the exchangeable hydrogens to the hydrogens deduced from the ^{13}C nmr spectrum we conclude that there are 34 hydrogens in compound **45**.

The high resolution mass spectrum of compound **45** does not show a molecular ion but displays fragment ions at m/z 250, 248 and 232. The cims shows an ion at m/z of 498 which agrees with a molecular formula of $C_{28}H_{34}O_8$ for compound **45**. Furthermore, the ions at m/z 248 and 250 observed in the hreims add together to give this molecular formula.

The arrangement of the various groups observed in the ^{1}H nmr spectrum of compound **45** was investigated by the use of nOe difference experiments*. The methine at δ 3.63 ppm gave nOe enhancement to the methyls hydrogens at δ 1.26 (4%) and 1.42 ppm (4%), and to a hydrogen of the sorbyl chain at δ 6.49 ppm (18%). The methylene hydrogen at δ 2.44 ppm gave an nOe enhancement to a hydrogen of the other sorbyl chain at δ 6.39 ppm (7%), to the methylene hydrogen at δ 2.71 ppm (12%), and to the methyl hydrogens at δ 1.19 ppm (2%). Furthermore, the methyl hydrogens at δ 1.19 ppm gave enhancement to the methylene hydrogen at δ 2.44 ppm (1.4%) and the methyl hydrogens at δ 1.24 ppm to the sorbyl chain hydrogen at δ 6.39 ppm (1.4%). These results allow us to conclude that two methyl groups and a sorbyl chain are in close proximity to the methine hydrogen, while the methylene group is close to a methyl group and another sorbyl chain. This indicates the presence of fragments **46** and **47** in compound **45**. The methyl hydrogens at δ 1.40 ppm did not show nOe enhancement to any hydrogen.

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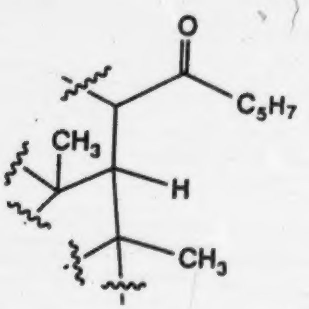
* The nOe experiments were performed in MeOH-d_4 at 200 MHz, therefore the chemical shifts mentioned are in MeOH-d_4



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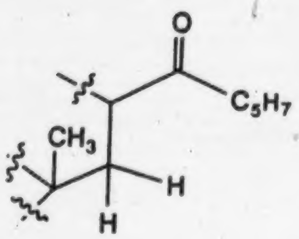

47

The similarity in molecular formula and the functional groups in compound **45** and the molecular formula and functional groups in trichodimerol (**11**) suggests that the two are closely related. Both compound **45** and trichodimerol have two sorbyl chains, four methyl groups as singlets in the ^1H nmr spectrum, and two chelated enols. Both compound **45** and trichodimerol have 28 carbons and 8 oxygens and are highly unsaturated. Furthermore the CD spectrum of compound **45** is similar to the CD spectrum of trichodimerol (**11**). The CD of compound **45** shows extrema of opposite sign at 410 and 353 nm (A = -75) corresponding to a negative chirality. This indicates the presence of two chromophores in compound **45** which are disymmetrically located relative to one another.

A compound called bisvertinol had been isolated previously from the culture broth of *V. intertextum* by Dreiding and co-workers (21d). Comparison of the spectral data of compound **45** obtained from *T. longibrachiatum* and the reported spectral data of bisvertinol isolated from *V. intertextum* showed that the two compounds are identical.

Dreiding and co-workers proposed structure **48** for bisvertinol with a *trans* stereochemistry between the H-9a methine and the Me-17 but no stereochemical assignment at C-4a or C-9b*. This lack of stereochemical information for these centers precluded them from determining the relative stereochemistry of bisvertinol. However, they proposed the absolute stereochemistry of C-4 and C-5a, as shown in **48**, on biogenetic grounds (21d). The results of our nOe experiments and the CD spectrum indicate that the relative stereochemistry of Me-17, H-9a and Me-25 in bisvertinol proposed by Dreiding and co-workers should be revised to an all *cis* stereochemistry. The stereochemistry of the C-4a hydroxyl should be *cis* on the basis of the enhanced stability of a *cis* 5-6 ring junction over a *trans* 5-6 ring junction, while the stereochemistry at C-4 should remain the same on biogenetic arguments. The structure of bisvertinol should thus be revised to **45**.

The CD spectrum of bisvertinol indicates that its absolute stereochemistry is as shown in **45**. In order to assign the absolute stereochemistry of bisvertinol by use of the exciton coupling method, knowledge of the relative direction of the electric transition dipole moment vectors of the chromophores in bisvertinol is required. After considering the chromophores in bisvertinol we conclude that the electric transition dipole moment vectors of the chromophores are aligned along the long axis of the sorbyl chains. Examination of molecular models shows that in order to observe a negative chirality in the CD spectrum the absolute stereochemistry of bisvertinol should be as shown in **45**.

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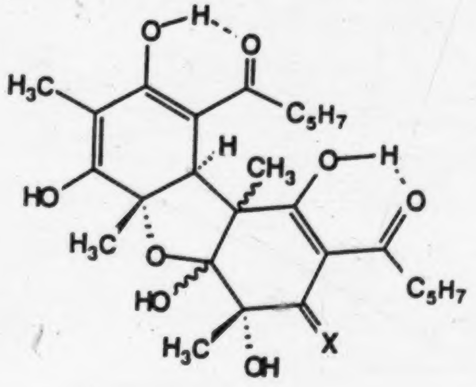
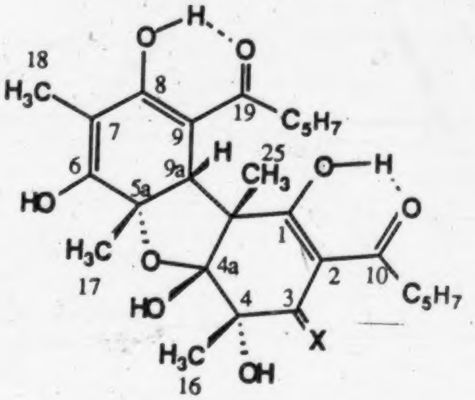
* Dreiding numbering system (21d).

The absolute stereochemistry of bisvertinol is consistent with the absolute stereochemistry of trichodimerol, which we have determined, and with the absolute stereochemistry of vertinolide (**49**), isolated from *V. intertextum* (21a, 21b), the absolute stereochemistry of which has been determined by total synthesis (23).


O–H
O
H_3C
C_5H_7
H
CH_3
O–H
O
HO
H_3C
O
C_5H_7
HO
H_3C
X
OH

48 $X = H_2$
51 $X = O$


O–H
O
18
H_3C
8
19
C_5H_7
7
9
9a
H
25
CH_3
O–H
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5a
HO
1
O
H_3C
17
O
4a
2
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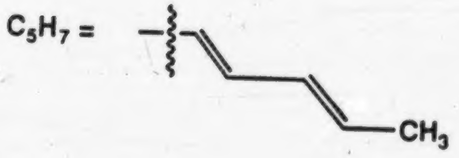
C_5H_7
HO
4
3
X
H_3C
16
OH

45 $X = H_2$
50 $X = O$



Bisvertinolone (50)

When *T. longibrachiatum* is grown on shake culture, a second yellow pigment, compound **50**, different from bisvertinol, is produced. The ir spectrum of compound **50** shows absorption for hydroxyl (3400 cm^{-1}), and a strongly chelated carbonyl (1663 cm^{-1}). Its uv spectrum shows maxima at 366 ($\varepsilon = 24000$), 294sh, and 272 (23000) nm which shift to longer wavelengths upon addition of base (394 and 276 nm) thus indicating that the chromophores in compound **50** have acid properties.

The ^{1}H nmr spectrum of compound **50** shows great similarities to the ^{1}H nmr spectrum of bisvertinol (**45**). As in the ^{1}H nmr spectrum of bisvertinol, the ^{1}H nmr spectrum of compound **50** shows signals corresponding to methyl hydrogens (δ 1.35, 1.42, 1.43, and 1.46 ppm), two sorbyl chains {δ 1.86 (d, 7 Hz, 3H), 1.88 (d, 7 Hz, 3H), 6.0-6.4 ppm (m, 6H), 7.31 (dd, 15, 10 Hz, 1H), 7.56 (dd, 15, 10 Hz, 1H), 7.39 (d, 15 Hz, 1H)}, hydroxyl hydrogens (δ 4.20 and 4.54 ppm), chelated enol hydrogens (δ 16.31 and 17.71 ppm), and a methine hydrogen (δ 3.72 ppm). However, the ^{1}H nmr spectrum of compound **50** does not show the presence of a methylene as observed in the ^{1}H nmr spectrum of bisvertinol (**45**).

The ^{13}C nmr spectrum of compound **50** is similar to the ^{13}C nmr spectrum of bisvertinol (**45**). The ^{13}C nmr spectrum of compound **50** shows signals assigned to six methyl carbons, a methine carbon (δ 54.4 ppm), a quaternary carbon (δ 59.8 ppm), tertiary carbons bearing oxygen (δ 79.0 and 79.8 ppm), eight sp^2 carbons as doublets, an hemiacetal carbon (δ 99.8 ppm), electron rich sp^2 carbons as singlets (δ 104.0, 107.2, and 110.9 ppm), and several carbonyl like carbons (δ 163.8, 170.2, 185.7, 191.2, 196.4, and 199.8 ppm). The main difference in the ^{13}C nmr spectrum of **50** when compared with the ^{13}C nmr spectrum of bisvertinol (**45**) is the absence of the methylene carbon signal at δ 36.5 ppm.

The differences in the ^{1}H and ^{13}C nmr spectra of bisvertinol and compound **50** indicate that compound **50** is an oxo derivative of bisvertinol, i.e., the methylene in **45** has been replaced by a ketone.

Further comparison of the ^{1}H nmr spectra of bisvertinol (**45**) and compound **50** corroborates the structural assignment of compound **50**. Comparison of the ^{1}H nmr chemical shifts reveal that H-11 shifted downfield from approx. δ 6.4 ppm in bisvertinol (**45**) to δ 7.39 ppm in compound **50** (approx. 1 ppm). This shift is reasonable since H-11 would lie in the deshielding region of the ketone at C-3. Comparison of the spectroscopic data of compound **50** with the published data for bisvertinolone, another compound isolated from the culture broth of *V. intertextum* (21d), indicates that the two compounds are identical.

Dreiding and co-workers proposed the structure **51** for bisvertinolone. However, nOe experiments performed by us (see Experimental) indicate that the H-9a methine and the Me-17 should be *cis*. The methine at δ 3.72 ppm gives nOe enhancement with the methyls at δ 1.42 and 1.43 ppm and to the sorbyl chain hydrogen at δ 6.38 ppm while the methyl hydrogens at δ 1.35 and 1.46 ppm did not show nOe enhancement of any hydrogen. Accordingly we propose structure **50** for bisvertinolone.

The CD spectrum of bisvertinolone (**50**) shows extrema of opposite sign at 402 and 350 nm (*A* = -42.2) corresponding to a negative chirality. This indicates that the absolute stereochemistry of bisvertinolone is as shown in **50**. Examination of molecular models shows that in order to observe a negative chirality in the CD spectrum the absolute stereochemistry of bisvertinolone should be as shown in **50**. This is consistent with the absolute stereochemistry of the other compounds in the series.

Bislongiquinolide (52)

Bislongiquinolide (**52**) is isolated from the broth extract of *T. longibrachiatum* (UAMH 5068). It is an optically active syrup {$[\alpha]_D$ +105° (*c* 0.55, MeOH)}. Its ir spectrum shows absorption for hydroxyl (3400 cm^{-1}), ketonic carbonyl (1732 cm^{-1}), strongly chelated carbonyl (1660 and 1626 cm^{-1}), and double bond groups (1602 cm^{-1}). The maxima in the uv spectrum of bislongiquinolide (**52**) (262 (ε = 18800), 291 (19800), 370 (15300), 385sh nm) shifts to longer wavelengths upon addition of base (204, 259, 287, and 393 nm), and to shorter wavelengths upon addition of acid (232, 294, 368, and 385 nm) indicating that the chromophores present have both weak and strong acid characteristics.

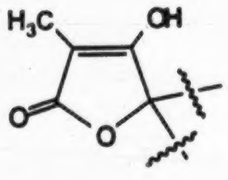
The molecular formula of bislongiquinolide, $C_{28}H_{32}O_8$, is obtained from the hreims and confirmed by cims. The mass spectrum shows fragment ions corresponding to the loss of water (m/z 478), the loss of CO (m/z 468), the loss of a sorbyl chain (m/z 401), the consecutive loss of CO and a sorbyl chain (m/z 373), and at m/z 95 (C_6H_7O) corresponding to the sorbyl chain.

The ^{1}H nmr spectrum displays methyl singlets (δ 1.10, 1.28, 1.49, and 1.56 ppm), signals corresponding to two sorbyl chains, an enolized β-diketone system {δ 14.02 (br. s, 1H) ppm}, a three hydrogen spin system {δ 3.18 (dd, 2, 6 Hz, 1H), 3.34 (d, 2 Hz, 1H), and 3.39 (d, 6 Hz, 1H) ppm}, and hydroxyl hydrogens {δ 5.00 (br. s, 1H), around δ 10 (v. br. s, 1H) ppm}. The stereochemistry of the sorbyl substituents is assigned as E,E on the basis of the *trans* coupling constant (15 Hz) of the signals at 7.23 and

7.34 ppm, and the similarity of this compound to the other compounds in the series.

The ^{13}C nmr spectrum of bislongiquinolide shows absorptions for methyl carbons (δ 6.3, 11.1, 19.0, 19.3, 23.2, and 23.5 ppm), methine carbons (δ 42.4, 43.6, and 51.3 ppm), a quaternary carbon (δ 62.6 ppm), tertiary carbons bearing oxygen (δ 74.5 and 82.9 ppm), electron rich sp^2 carbons (δ 98.2 and 108.5 ppm), eight sp^2 carbons as doublets, and several carbonyl like carbons (δ 169.7, 174.2, 176.1, 194.9, 202.6, and 208.2 ppm). The tertiary carbon bearing oxygen at δ 82.9 ppm is low field, suggesting that the oxygen is acylated (49). The methyl carbon at δ 6.3 ppm is high field indicating that it is in a sterically crowded environment. Sterically hindered aromatic methyl signals are also observed at high field in the ^{13}C nmr spectra of sorbicillin (**1**, 7.0 ppm) and sorbiquinol (**37**, 7.6 ppm).

Comparison of the spectroscopic properties of bislongiquinolide (**52**) with those of vertinolide (**49**), a tetronic acid isolated from *V. intertextum* (21b), and other tetronic acids (81), suggest the presence of a tetronic acid functionality[*] (**53**) in bislongiquinolide (**52**).



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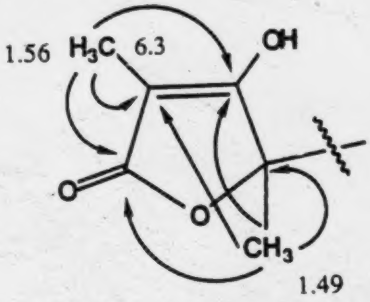
[*] Tetronic acids are known to have pKa's in the order of 5 (see reference 21b and references therein).

In the ^{1}H nmr spectrum of tetronic acids the hydroxyl hydrogen appears around δ 10 ppm, as a very broad signal (21b, 82) and in the ^{13}C nmr spectrum the methyl signal appears at δ 6-7 ppm (81).

The nmr spectra of bislongiquinolide and vertinolide (**49**) show important similarities. The ^{13}C nmr spectrum of vertinolide (**49**) shows absorptions at δ 6.2 (q), 23.6 (q), 82.6 (s), 97.2 (s), 173.9 (s), and 176.6 (s) corresponding to the tetronic acid fragment (**54**). The chemical shifts of these signals compare favorably with the signals observed for bislongiquinolide at δ 6.3 (q), 23.2 (q), 82.9, 98.2 (s), 174.2 (s), and 176.1 (s) ppm. In addition, the ^{1}H nmr spectra of bislongiquinolide (**52**) and vertinolide (**49**) show similarities. The methyl hydrogens (Me-13 and Me-14) in vertinolide appear at δ 1.48 and 1.68 ppm and the hydroxyl of the tetronic acid at δ 9.80 ppm, while in bislongiquinolide there are signals for methyls at δ 1.49, 1.56 ppm and for a hydroxyl at 10.0 ppm.

In order to confirm the presence of a tetronic acid in bislongiquinolide we performed a series of selective INEPT experiments. Selective irradiation of the methyl hydrogens at δ 1.56 ppm gave signals at δ 98.2, 174.5, and 176.3 ppm, while irradiation of the methyl hydrogens at δ 1.49 ppm gave carbon signals at δ 83.0, 98.2, 174.5, and 176.2 ppm. The selective INEPT (INAPT) experiments thus allow the assignment of the signals in the ^{1}H nmr spectrum at δ 1.56 and 1.49 ppm to the methyl on the electron rich carbon of the tetronic acid and to the methyl on the carbon bearing oxygen, respectively. These results are consistent with the presence of the tetronic acid fragment **54** in bislongiquinolide (**52**) and allow the assignment of the ^{13}C nmr chemical shifts of the carbonyl carbons at δ 174.5 and 176.3 ppm and the electron rich carbon at δ 98.2 ppm to this fragment.



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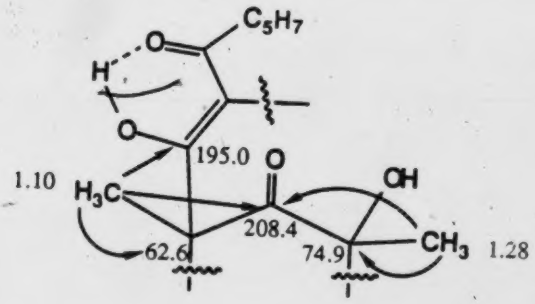
Further assignments of the carbonyl signals observed at δ 169.7, 195.0, 202.7, and 208.4 ppm in the ^{13}C nmr of bislongiquinolide were made on the basis of chemical shift. The signals at δ 169.7 and 195.0 may be assigned to the enolized β-diketone system, and the signals at δ 202.7 and 208.4 to the sorbyl chain carbonyl and a ketone carbonyl, respectively. The presence of an alcohol hydroxyl hydrogen in the ^{1}H nmr spectrum of bislongiquinolide allow us to conclude that the carbon at δ 74.9 ppm corresponds to a tertiary alcohol. Since all the carbonyl signals in the ^{13}C nmr spectrum have been assigned, the enolized β-diketone system should be part of an enol-sorbyl system, as we have seen in previous compounds.

The molecular formula of bislongiquinolide indicates it has thirteen unsaturations. Three unsaturations may be attributed to one sorbyl chain, four to the sorbyl-enol system, three to the tetronic acid, and one to the ketone observed in the ^{13}C nmr (δ 208.2 ppm). Two unassigned unsaturations remain, indicating that bislongiquinolide is bicyclic. The chelated enol, the tetronic acid, the two sorbyl chains, the tertiary alcohol, and the ketone account for all of the oxygens in the molecular formula of **52**.

The INAPT experiments furnished further structural information. Selective irradiation of the methyl hydrogens at δ 1.10 ppm gave signals in the ^{13}C nmr for the quaternary carbon, the ketone carbonyl, and the chelated enol system carbon at δ 195.0, while irradiation of the methyl hydrogens at δ 1.28 ppm gave signals for the tertiary carbinol carbon and the ketonic carbon. These results allow us to derive fragment **55**, which accounts for the observed INAPT correlations.



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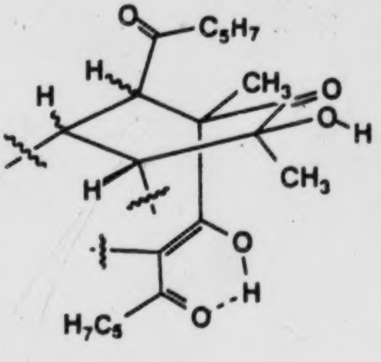
Three different series of nOe experiments (360 MHz) gave information on the relative distribution of the substituents in bislongiquinolide. The series of nOe experiments were carried out under the following conditions: at room temperature in a benzene/chloroform mixture, where the nOe enhancements observed in this case were all negative; at 318 K in chloroform, where the nOe enhancements observed were positive and small; and in chloroform at dilute concentration and room temperature, where no nOe enhancements were observed! However the nOe experiments in a $CDCl_3$/benzene-d_6 mixture at room temperature gave the following results: Irradiation of the methyl hydrogens at δ 1.28

ppm gave enhancement to the methine hydrogen at δ 3.34 ppm (-2%) while irradiation of the methine hydrogen at δ 3.34 ppm gave enhancemen to the sorbyl chain hydrogen at δ 6.2 ppm (d, 15 Hz) (-16%) and to th methine hydrogen at δ 3.18 (-10%). Irradiation of the methine hydrogen a δ 3.39 ppm gave enhancement to the methine hydrogen at δ 3.18 (-5%), th sorbyl chain hydrogen at δ 6.2 ppm (d, 15 Hz) (-8%), and the sorbyl chai hydrogen at δ 7.23 (dd, 15, 10 Hz) (-6%), while irradiation of the methin hydrogen at δ 3.18 ppm gave enhancement to the methines at δ 3.3 (-8.5%) and 3.39 ppm (-11%). Furthermore, irradiation of the methyl at 1.10 ppm gave enhancement to the sorbyl chain hydrogen at δ 6.2 ppr (-1.2%) and to the methine hydrogen at δ 3.39 ppm (-1%). These observations and the presence of fragment **55**, obtained from the INAP experiments, allow us to derive fragment **56** in bislongiquinolide.



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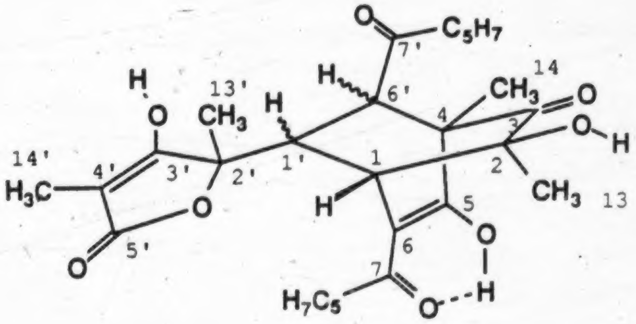
The nOe experiments at 318 K in chloroform gave the followin results: Irradiation of the methyl hydrogens at δ 1.28 ppm gave positiv nOe enhancement to the methine hydrogen at δ 3.34 ppm (1%), whil simultaneous irradiation of the methine hydrogens at δ 3.34 and 3.39 ppn gave enhancement to signals from the two sorbyl chains, at δ 6.12 and 6.16 ppm (13%), indicating that these two hydrogens are in close proximity to the sorbyl chains. The methyl hydrogens at δ 1.49 ppm gave enhancement with the three methine hydrogens at δ 3.18, 3.34, and 3.39 ppm and this is consistent with the presence of fragment **54** as a freely rotating group in bislongiquinolide. The nOe results in $CDCl_3$ at 318 K are similar to the nOe results obtained in $CDCl_3$/benzene-d_6 but of opposite sign.

The nOe enhancement experiments establish **57** as the relative structure of bislongiquinolide. Structure **57** explains all the spectral data of bislongiquinolide, however, the relative stereochemistry at carbons C-1', C-6', and C-2 cannot be determined on the basis of the nOe results.



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The apparently contradictory results obtained in the nOe experiments when the conditions are changed are explained by assuming that the extreme narrowing condition is not met. If the extreme narrowing condition is not met during nOe experiments, the value of the nOe enhancements may be low, zero or negative. The nOe enhancements depend on a balance between relaxation through zero quantum and double quantum pathways. However, if double quantum transitions dominate the relaxation of the spin system, the nOe enhancements observed are positive. If zero quantum transitions however, dominate the relaxation of the spin system, the nOe enhancements observed are negative (83). Under conditions where the extreme narrowing condition is not met, the two relaxation mechanisms have similar importance. This situation can, therefore, lead to the observation of zero, positive, or negative enhancements, depending on a number of factors. In the case of bislongiquinolide a benzene solution gave negative enhancements. When the temperature of a chloroform solution was raised the nOe enhancements observed were positive while at room temperature the nOe vanished!

The relative stereochemistry at C-2 may be assigned tentatively as shown in **52** on the basis of the ^{1}H nmr chemical shift of Me-13. A chemical shift of δ 1.28 ppm is high field for a methyl on a carbon bearing oxygen. For this reason we presume that Me-13 lies in the shielding region of the enolized β-diketone system, and therefore it is assigned as shown in **52**. Furthermore, the absolute stereochemistry of C-2' is assigned as shown in **52** on the basis of the similarity between bislongiquinolide and vertinolide* (**49**).

The CD spectrum of bislongiquinolide gives a complex pattern that does not resemble the two chromophore interaction. It does resemble, however, the theoretical spectra obtained by Harada and co-workers for a three chromophore system (84). Therefore, the absolute stereochemistry of bislongiquinolide cannot be assigned unambiguously. However, based on the structural similarity between bislongiquinolide, trichodimerol and bisvertinol, we tentatively assign the absolute stereochemistry of bislongiquinolide as shown in **52**.

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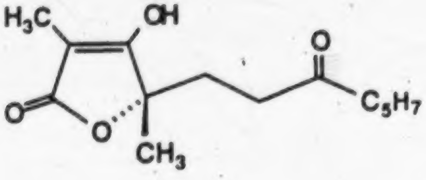
* Recall the absolute stereochemistry of vertinolide was determined by total synthesis (23).



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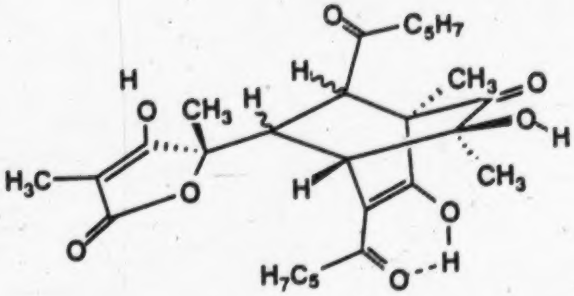

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5-Hydroxyvertinolide (58)

A second tetronic acid derivative (**58**) was isolated from the culture broth of *T. longibrachiatum* (UAMH 4159), and detected in the culture broth of *T. longibrachiatum* (UAMH 5068) as an optically active {$[\alpha]_D$ -64° (*c* 0.11, MeOH)} solid. Its uv spectrum shows absorptions at 230sh, 263 (ε = 12900), and 359 (900) nm, with maxima shifting upon addition of base (203, 260, and 373 nm), indicating that the chromophore has acidic properties. The ir spectrum of **58** shows the presence of hydroxyl (3360 cm^{-1}), carbonyl (1749 and 1666 cm^{-1}), and double bond (1638 and 1593 cm^{-1}) groups. The mass spectrum did not provide a molecular ion.

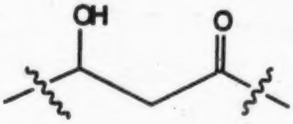
The ^1H nmr spectrum of compound **58** shows signals corresponding to methyls (δ 1.49 and 1.71 ppm), a hydroxyl (δ 3.49 ppm), a sorbyl chain, and a three spin system {δ 2.77 (dd, 16, 10 Hz, 1H), 3.15 (dd, 16, 2 Hz, 1H), and 4.14 (dd, 10, 2 Hz, 1H)}.

The ^{13}C nmr spectrum of compound **58** shows 14 carbons, signals corresponding to methyl carbons (δ 5.3, 18.1, and 18.1 ppm), a methylene carbon (δ 40.5 ppm), a secondary alcohol (δ 70.3 ppm), a tertiary carbon bearing oxygen (δ 82.8 ppm), four sp^2 carbons as doublets, and carbonyl like carbons (δ 173.4, 175.4, and 198.5 ppm).

The ^{13}C nmr data is consistent with a molecular formula with at least 14 carbons, 16 hydrogens, and 5 oxygens for compound **58**. There are two exchangeable hydrogens clearly seen in the ^1H nmr spectrum of compound **58**, and this information, together with the information from the ^{13}C nmr spectrum allows the conclusion that the molecular formula of compound **58** is $C_{14}H_{18}O_5$.

The presence of a methyl signal at δ 5.3 ppm in the ^{13}C nmr spectrum of compound **58** indicates the presence of a methyl in a sterically crowded environment. This observation and the presence of signals in the ^{13}C nmr spectrum at δ 173.4, 175.4, 82.8, and 96.4 ppm as singlets suggest the presence of a tetronic acid moiety in compound **58**.

The secondary alcohol observed in the ^{13}C nmr spectrum of compound **58** at δ 70.3 ppm is confirmed by the presence of a hydrogen on a carbon bearing oxygen at δ 4.14 ppm in the ^{1}H nmr spectrum of compound **58**. Since this hydrogen is part of a three spin system, the fragment **59** can be derived. The large coupling constant observed in the ^{1}H nmr spectrum of **58** between the hydrogens at δ 3.15 and 3.49 ppm (16 Hz) indicates that these hydrogens are geminally coupled and α to a carbonyl.



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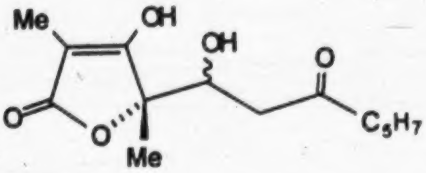
Only one carbonyl signal remains unaccounted for in the ^{13}C nmr of compound **58**, and this has to be assigned to the sorbyl carbonyl. The methylene of fragment **59** must be α to this carbonyl. This leaves only one point at which to attach the tetronic acid fragment. Thus the structure of compound **58** is assigned as 5-hydroxyvertinolide (**58**).

The relative stereochemistry of 5-hydroxyvertinolide cannot be determined with the information available.

The CD spectrum of 5-hydroxyvertinolide does not show strong Cotton effects and therefore indicates that compound **58** is not dimeric. Furthermore, both vertinolide and 5-hydroxyvertinolide have negative specific rotations {-64 (*c* 0.11, MeOH) and -25.0 (*c* 0.05, $CHCl_3$) (21b)} respectively). Unfortunately, Dreiding and co-workers did not report the CD spectrum of vertinolide. The similarity in structure of 5-hydroxyvertinolide and the other compounds from *T. longibrachiatum* with the metabolites from *V. intertextum*, and between the chromophores present in vertinolide and 5-hydroxyvertinolide, lead us to assign the absolute stereochemistry of C-4 in 5-hydroxyvertinolide as shown in **58**. The absolute stereochemistry of vertinolide is known from total synthesis (23).



58

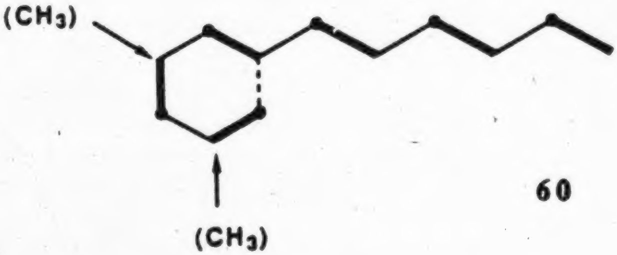
Biosynthesis of the metabolites of *Trichoderma longibrachiatum.*

Biogenetic considerations have been important in elucidating the structure and stereochemistry of the metabolites of *T. longibrachiatum.* For example, the relative stereochemistry of sorbiquinol at C-16 and its absolute stereochemistry; the relative stereochemistry at C-4 of bisvertinol and bisvertinolone; the stereochemistry at C-2', C-1', and C-2 of bislongiquinolide; and the absolute stereochemistry at C-4 of 5-hydroxyvertinolide are supported on biogenetic grounds. This importance and the postulated unusual dimerization and aromatic oxidation in the biosynthesis of the metabolites produced by *T. longibrachiatum* stimulated us to conduct biosynthetic experiments.

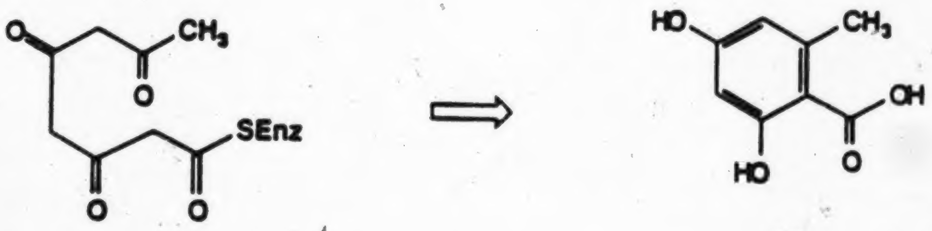
In order to obtain information on the biosynthesis of the metabolites of *T. longibrachiatum*, we performed ^{13}C incorporation experiments. Still cultures of *T. longibrachiatum* were fed with sodium [1-^{13}C]- and [1,2-$^{13}C_2$]acetate, the cultures were harvested (see Experimental), and the metabolites isolated. From the [1-^{13}C]acetate feeding we isolated sorbicillin, trichodimerol, trichodermolide, sorbiquinol (as tetrahydrosorbiquinol), bisvertinol, and bislongiquinolide, while only sorbicillin and trichodimerol were isolated from the [1,2-$^{13}C_2$]acetate experiment.

Biosysnthesis of sorbicillin.

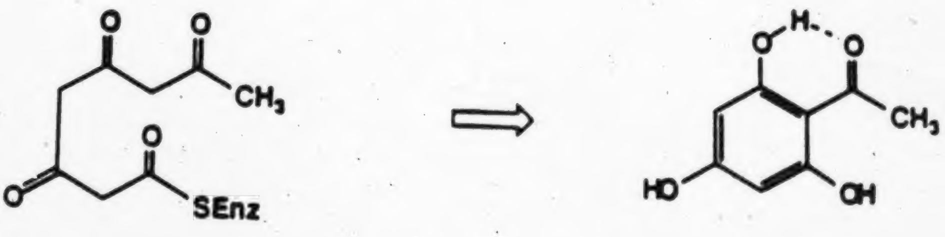
The structure of sorbicillin suggests that it is derived from acetate with the uncommon folding pattern shown in **60**. In the biosynthesis of the majority of the polyketides isolated from fungi (85) the condensation of the polyketide has occured by attack of the keto-ester stabilized anion to a ketone, rather than by attack of a diketo stabilized anion to the carboxylic end of the chain. The former condensation gives an orsellinic acid (**61**) type of compound, while the latter gives an acetylphloroglucinol (**62**) type of compound. Sorbicillin is of the latter class.



60

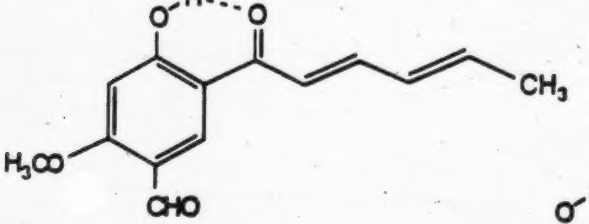


61

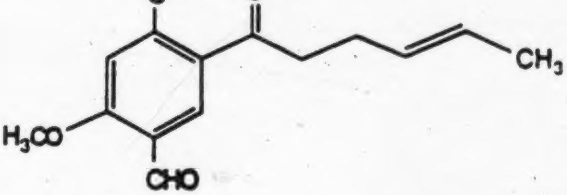


62

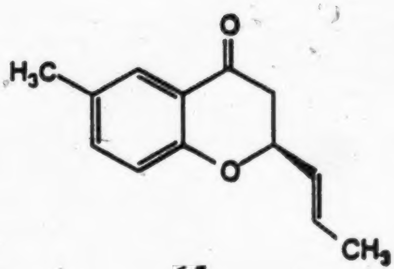
Other hexaketides with the same folding pattern of sorbicillin reported previous to 1983 are compounds **63**, **64**, and **65** (86).



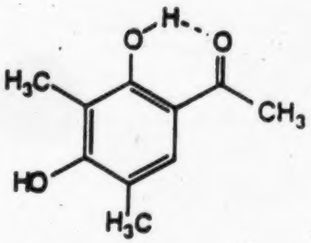
63



64



65



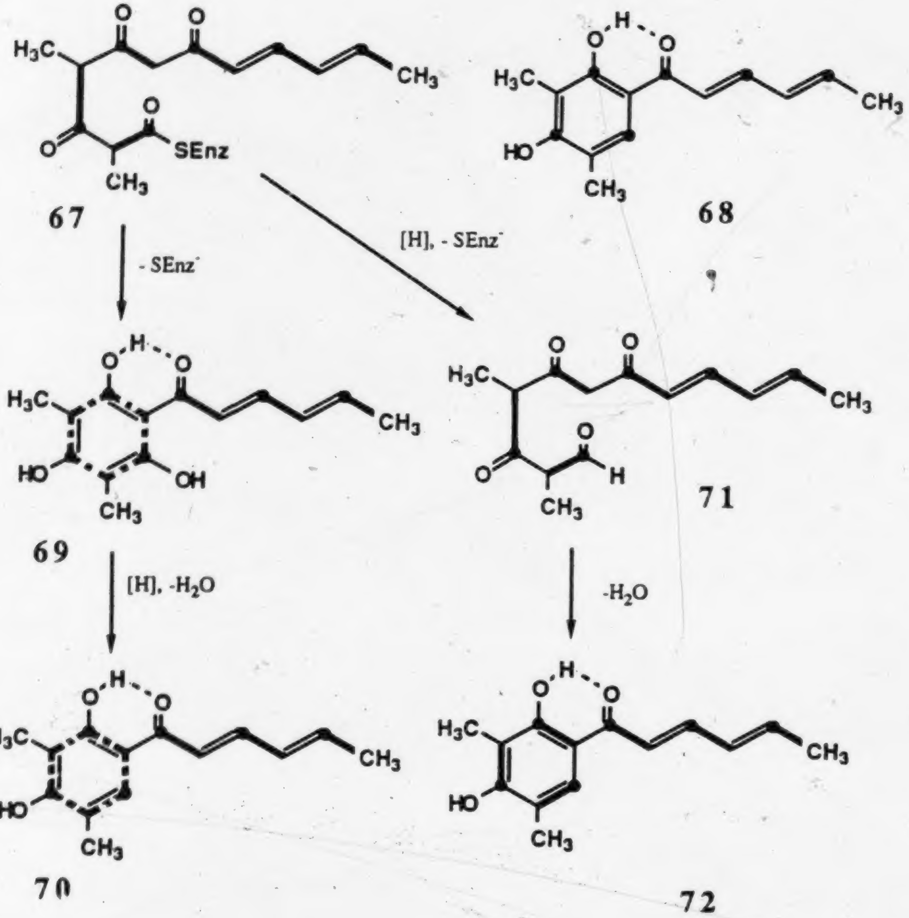
66

Turner speculates that: "...in the biosynthesis of clavatol (**66**) methylation occurs before cyclization and inhibits the more usual cyclization to a compound of the orsellinic acid type." (87). Such a mechanism may be true in the biosynthesis of sorbicillin. In this case cyclization of the methylated polyketide precursor (**67**) may occur to give sorbicillin, which is a compound of the acetylphloroglucinol type.

The ^{13}C nmr spectrum of [1-^{13}C]acetate incorporated sorbicillin shows that ^{13}C was incorporated (ca 20%) to the carbons at δ 128.2, 140.6, 144.0, 158.2, 162.0, and 192.0 ppm (Table 12). These carbon signals correspond to the carbons highlighted with a dot in structure **68**, and the results are consistent with the derivation of sorbicillin from acetate with the polyketide folding shown in **60**.

However, cyclization of the polyketide precursor of sorbicillin (**67**) may occur by two different routes (Scheme 2). Examination of the polyketide precursor (**67**), indicates that the carboxylic end is at the acid oxidation level, while in the product (**1**), it is at the "aldehyde" oxidation level. Therefore, a reduction of this carbon must occur before the formation of sorbicillin is complete. One route is the direct cyclization of precursor **67** to give the symmetrical compound **69**, followed by a deoxygenation to give sorbicillin. If the symmetrical intermediate **69** is a precursor to sorbicillin then scrambling of the labeling pattern of sorbicillin obtained from [1,2-$^{13}C_2$]acetate experiments, as illustrated in **70**, should be observed. This allows us to determine whether compound **69** is an intermediate in the production of sorbicillin. Another biosynthetic route for the production of sorbicillin requires the reduction of the thioester in **67** to an aldehyde, as shown in **71**, followed by condensation to form sorbicillin. If this is the case then sorbicillin will be the first enzyme free product and will have the labelling pattern shown in **72**.

Analysis of the ^{13}C nmr spectrum of [1,2-$^{13}C_2$]acetate derived sorbicillin shows no scrambling of the label. This result allows us to eliminate the possibility that a symmetrical intermediate such as **69** is a precursor of sorbicillin.


67
68
- SEnz⁻
[H], - SEnz⁻
69
71
[H], -H₂O
-H₂O
70
72

Scheme 2

Formation of Sorbicillin (**1**)

Assignment of the ^{13}C nmr spectrum of sorbicillin.

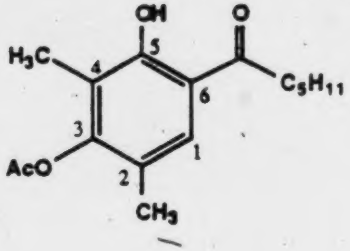
To prove the mechanism of formation of sorbicillin we studied the product derived when *T. longibrachiatum* was grown on sodium [1,2-$^{13}C_2$]acetate enriched media. However, in order to interpret the results it was necessary to assign the ^{13}C nmr spectrum of sorbicillin.

Some signals of the ^{13}C nmr spectrum of sorbicillin are readily assigned. The sorbyl chain carbons, the aromatic methyl carbons, and the aromatic methine carbons can be identified by chemical shift considerations together with the multiplicity of these signals, as observed in the APT spectrum. The assignment of the other carbons was determined in the following way. There are two carbons bearing oxygen, at δ 158.2 and 162.0 ppm, and three non-hydrogen bearing carbons, at δ 109.9, 113.0, and 113.9 ppm. Comparison of the ^{13}C nmr spectrum of sorbicillin (**1**) with that of tetrahydrosorbicillin (**3**) indicates that the shifts caused by the hydrogenation are not large enough to allow an unambiguous assignment of these carbons (at δ 158.8, 161.4, 110.4, 112.9, and 114.6 ppm in **3**). Therefore we prepared O-acetyl-tetrahydrosorbicillin (**73**) and compared its ^{13}C nmr spectrum with that of tetrahydrosorbicillin (**3**). The high field oxygen-bearing carbon was shifted upfield, from δ 158.8 ppm in **3** to δ 153.6 ppm in **73**, and this is assigned to C-3 in sorbicillin and tetrahydrosorbicillin.

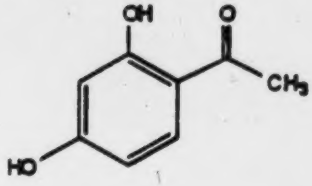
In order to assign the non-hydrogen bearing carbons, the ^{13}C nmr data of tetrahydrosorbicillin was compared with the reported values for 2,4-dihydroxyacetophenone (**74**) (88). The high field carbon bearing hydrogen (at δ 102.6) of 2,4-dihydroxyacetophenone was assigned to the carbon with two ortho hydroxyl groups*. On this basis, the high field non-hydrogen bearing carbon in tetrahydrosorbicillin and sorbicillin was assigned to C-4 .

[1,2-$^{13}C_2$]-Acetate Incorporation Into Sorbicillin (**1**).

The results of the [1,2-$^{13}C_2$]acetate incorporation (Table 13) show that the sp^2 carbon doublet at δ 128.2 ppm is coupled (63 Hz) only with the carbon at δ 113.9 ppm. These results are consistent with couplings between sp^2 and sp^2 carbons (55-70 Hz) and sp^2 and sp^3 carbons (43 Hz) and with the labelling pattern shown in **72**.



73



74

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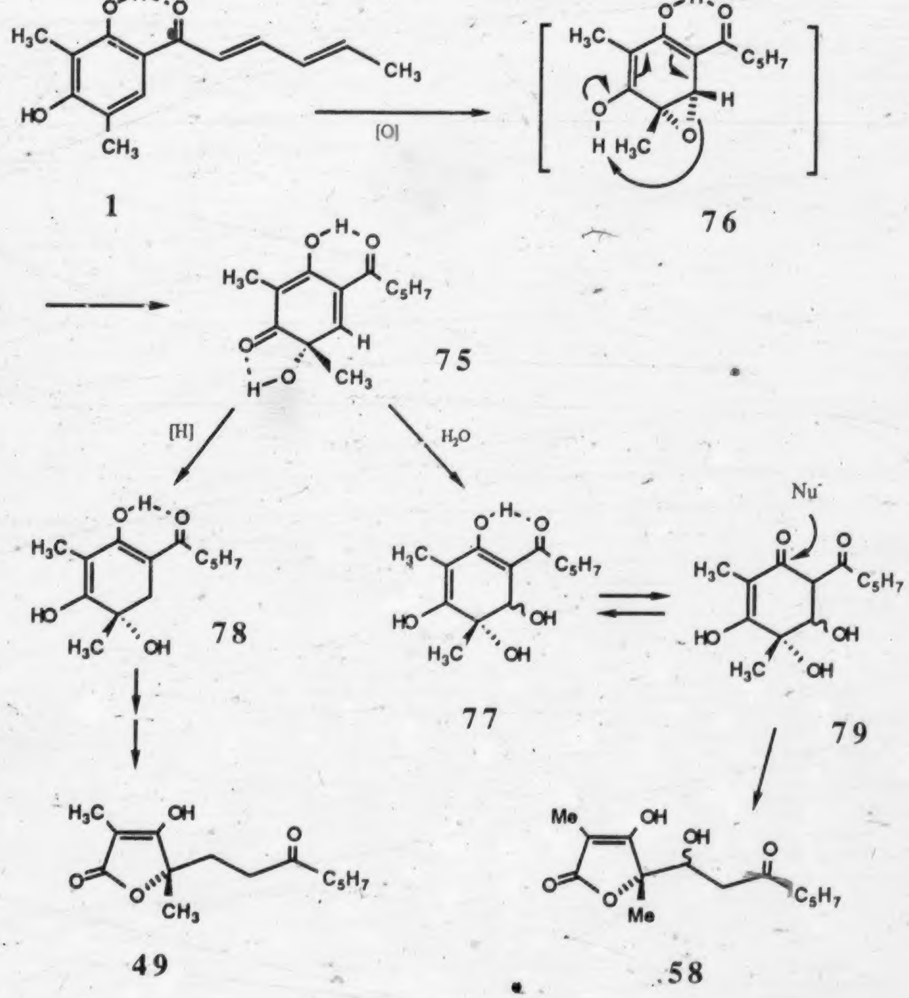
* The acyl bearing carbon is at δ 112.8 ppm in **74**.

Biosynthesis of 5-hydroxyvertinolide, bisvertinol, bisvertinolone, trichodimerol, sorbiquinol, and bislongiquinolide.

The biosynthesis of trichodimerol (**11**), bisvertinol (**45**), sorbiquinol (**37**), bislongiquinolide (**52**), and 5-hydroxyvertinolide (**58**) by *T. longibrachiatum* may proceed from sorbicillin through a common intermediate precursor, quinol **75** (Scheme 3). Aromatic epoxidation (89) of sorbicillin to produce **76**, followed by opening of the epoxide by the enol, gives rise to quinol **75**. Since aromatic epoxidations are known to occur to give optically active epoxides (90) it is likely that the absolute stereochemistry of the epoxide in an intermediate such as **76** determines the absolute stereochemistry of the metabolites of *T. longibrachiatum*.

Addition of water or hydride to quinol **75** gives rise to **77** and **78**, respectively, whereas tautomerization of **77** to **79** followed by a nucleophilic cleavage and lactonization yields 5-hydroxyvertinolide (**58**) (Scheme 3). The same reaction sequence with intermediate **78** produces the known compound vertinolide (**49**), isolated from *V. intertextum* (21b). The conversion of an intermediate like **77** to 5-hydroxyvertinolide (**58**), or **78** to vertinolide (**49**), may be enzymatic, since there is evidence that enzymes are involved in keto-enol tautomerizations (91).

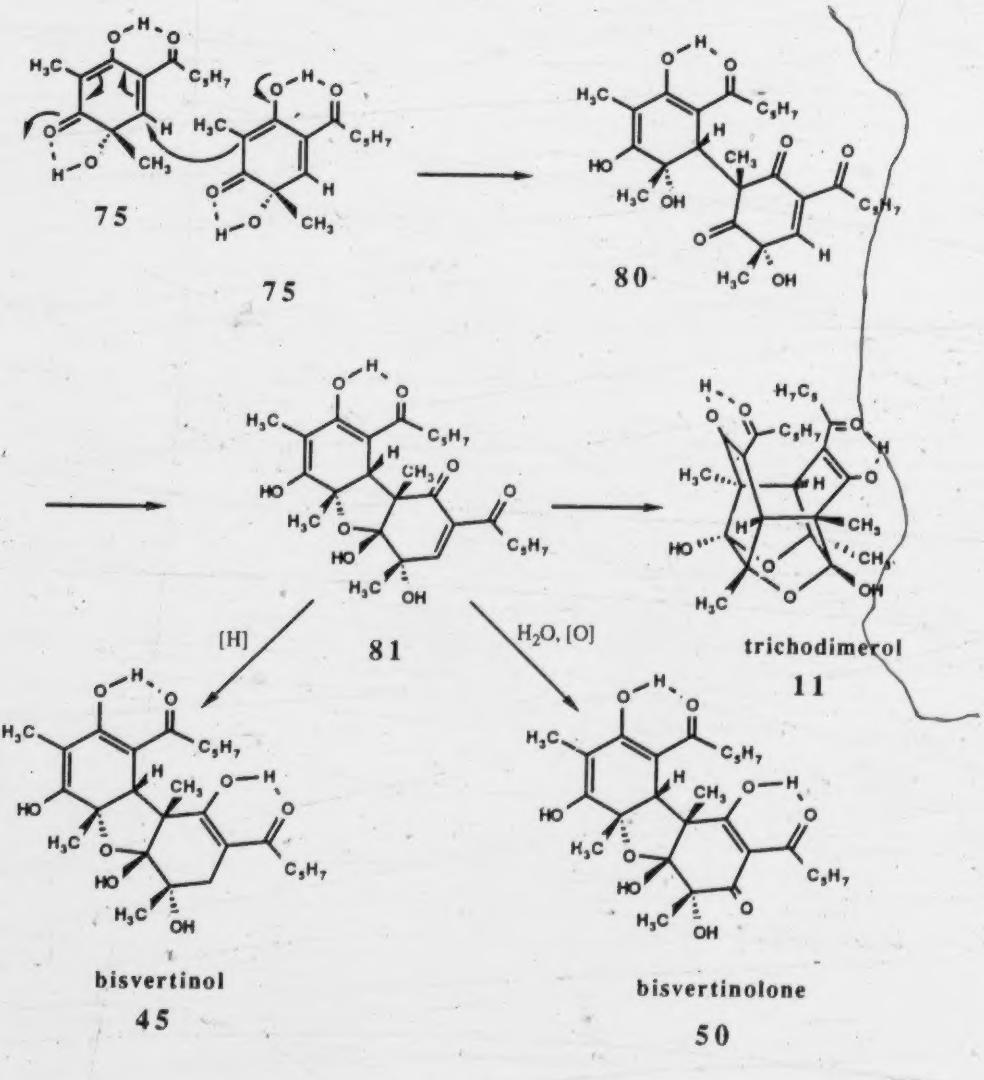

[O]
1
76
75
[H]
H_2O
Nu⁻
78
77
79
49
58

Scheme 3

Formation of vertinolide (**49**) and 5-hydroxyvertinolide (**58**) from a common intermediate.

Intermediate **75** can follow a different reaction course, dimerising as shown in Scheme 4, to give bisvertinol, bisvertinolone, or trichodimerol. Condensation of two molecules of intermediate **75** to give intermediate **80**, followed by hemiacetal formation gives intermediate **81**. Intermediate **81** may be the precursor to several metabolites. Addition of water to **81**, followed by oxidation gives bisvertinolone (**50**), while reduction of **81** gives bisvertinol (**45**). On the other hand internal C-C bond formation in **81**, followed by hemiacetal formation gives trichodimerol (**11**).

The ^{13}C nmr spectra of [1-^{13}C]acetate incorporated bisvertinol and trichodimerol show the incorporation of ^{13}C into the predicted carbons (Table 12), as shown in **82** and **83**. Furthermore, the ^{13}C nmr spectrum of [1,2-$^{13}C_2$]acetate incorporated trichodimerol shows that all acetate units remained intact as shown in **84**. The presence of intact acetate units was confirmed by a 2D INADEQUATE (46) spectrum of the [1,2-$^{13}C_2$]acetate incorporated trichodimerol (Figure 13). This spectrum shows connectivities between the carbons at δ 57.5 (d) and 78.7 ppm, δ 104.0 and 58.8 (s) ppm, δ 197.9 and 102.5 ppm, δ 143.0 and 131.7 ppm, δ 175.7 and 118.3 ppm, and δ 140.1 and 18.9 ppm. The connectivities and the C-C coupling constants are summarized in Table 13. These results are consistent with the hypothesis that bisvertinol and trichodimerol arise from sorbicillin.


75
75
80
81
[H]
H2O, [O]
trichodimerol
11
bisvertinol
45
bisvertinolone
50

Scheme 4

Formation of trichodimerol (**11**), bisvertinol (**45**), and bisvertinolone (**50**) from a common intermediate, quinol (**75**).

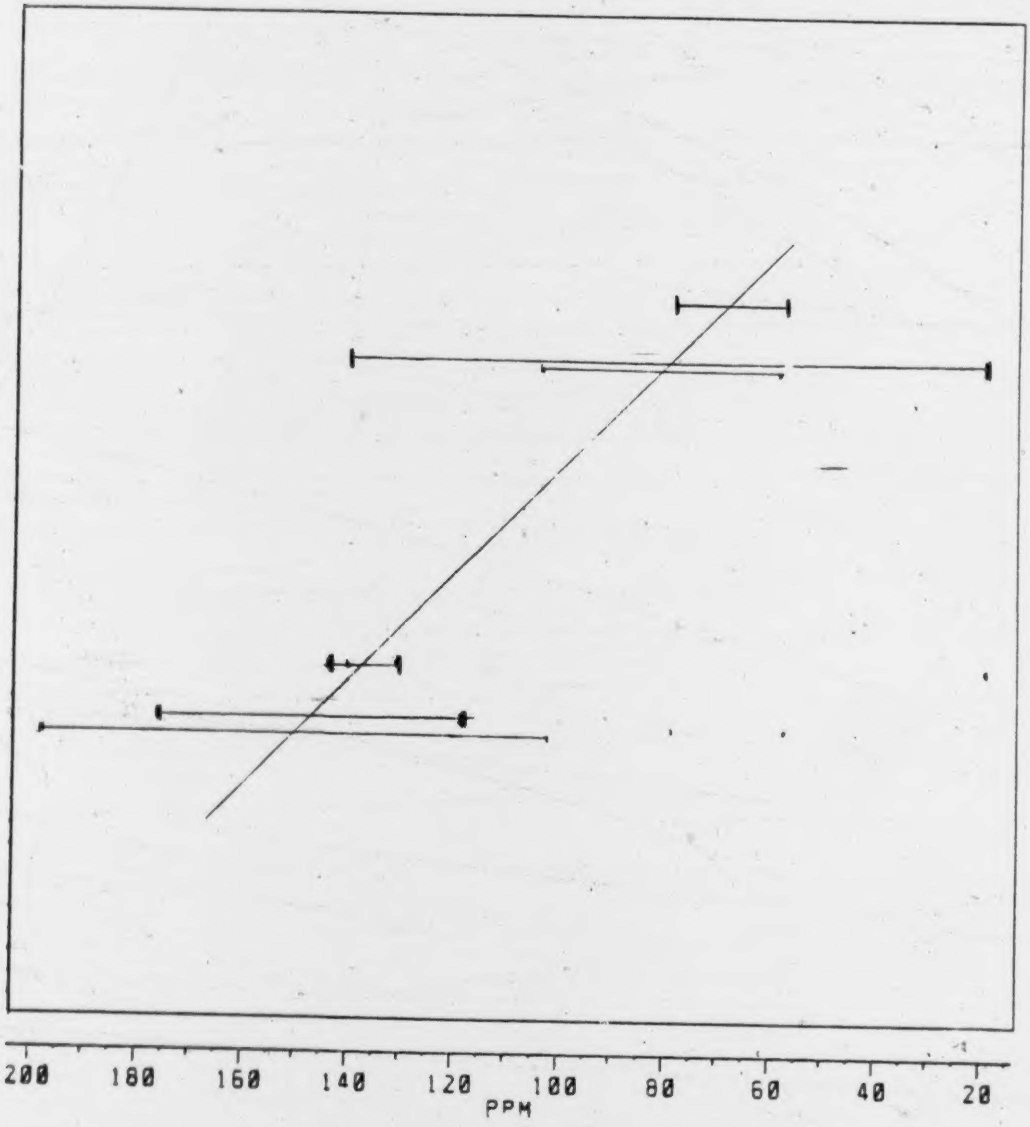
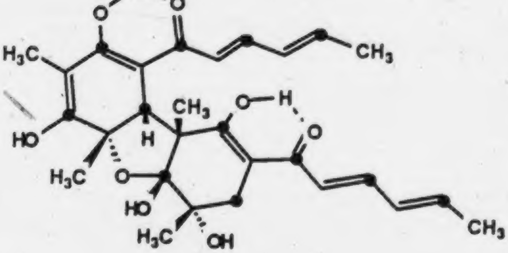
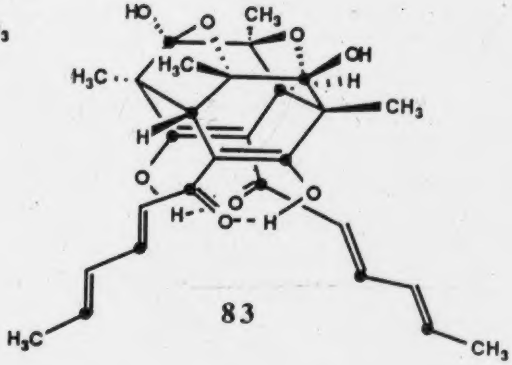


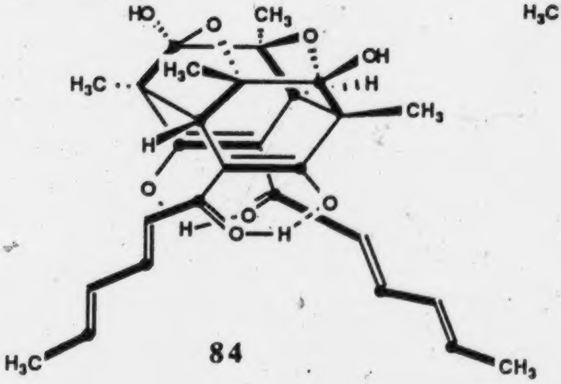
Figure 13. INADEQUATE 2D spectrum of [1,2-$^{13}C_2$]acetate incorporated trichodimerol (**11**).



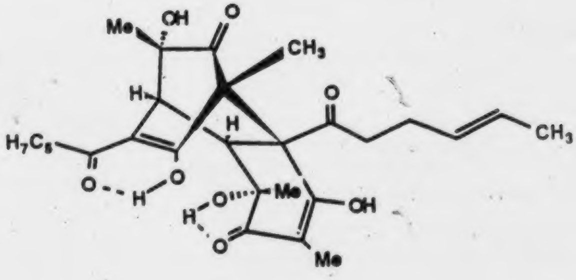
82



83



84

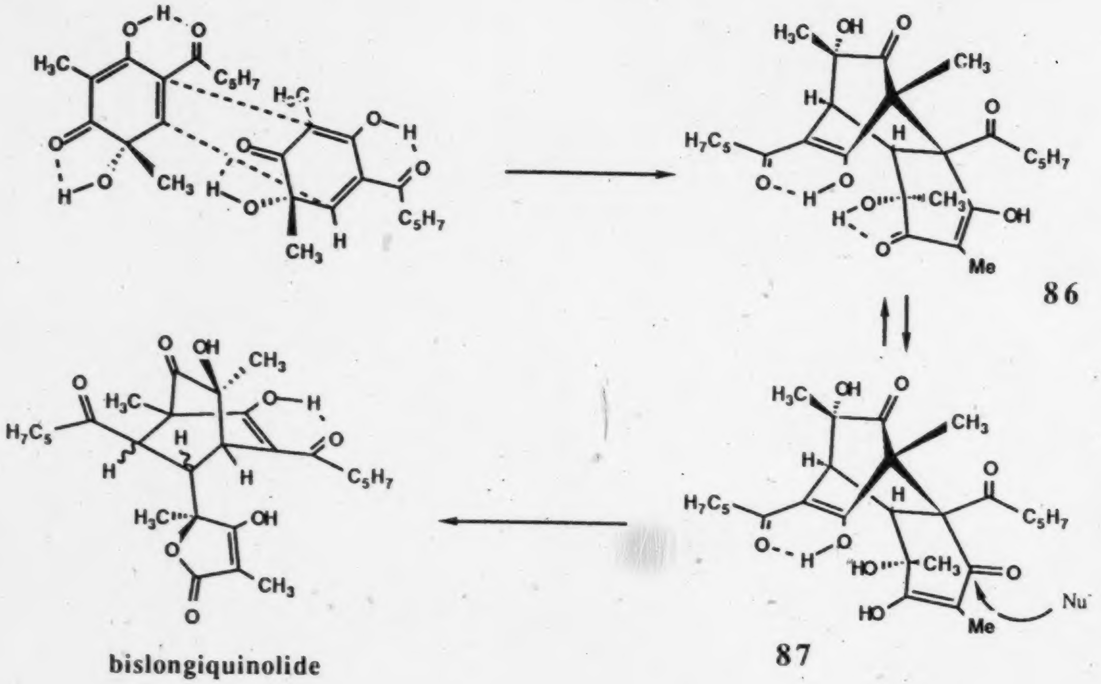


85

It is known that o-quinols like **75** dimerize readily (92, 93), one molecule of quinol acting as diene, the other as dienophile. Such dimerizations occur with a high degree of regio- and stereospecificity (92). This reaction may explain the formation of bisvertinoquinol (**85**) by *V. intertextum*, as indicated by Dreiding and co-workers (21c).

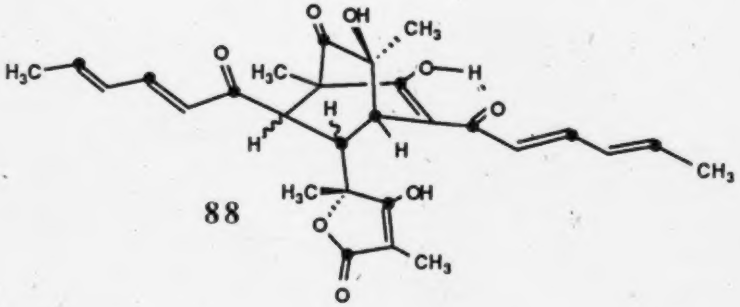
The isolation of bisvertinoquinol from *V. intertextum*, is interesting since it has the same carbon skeleton as dehydrobisvertinoquinol (**86**), a possible intermediate in the formation of bislongiquinolide (produced by *T. longibrachiatum*). As shown in Scheme 5, tautomerization of dehydrobisvertinoquinol (**86**) to **87**, followed by a nucleophilic cleavage and lactonization gives bislongiquinolide. This reaction sequence is similar to the one previously described for 5'-hydroxyvertinolide (**49**). Accordingly, the ^{13}C nmr spectrum of [1-^{13}C]acetate incorporated bislongiquinolide shows incorporation (approx. 20%) of ^{13}C into the carbons (Table 12) shown in **88**.

The formation of sorbiquinol by *T. longibrachiatum* may arise in a similar way. A Diels Alder reaction between the quinol **75** and sorbicillin would give sorbiquinol. The ^{13}C nmr spectrum of [1-^{13}C]acetate incorporated tetrahydrosorbiquinol shows incorporation of ^{13}C into the predicted carbons (Table 12) as shown in **89**. This is consistent with the hypothesis that sorbiquinol arises from sorbicillin and intermediate **75**.

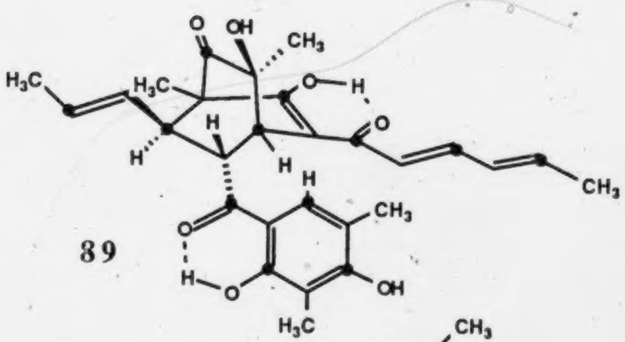

86
87
Nu⁻
bislongiquinolide

Scheme 5

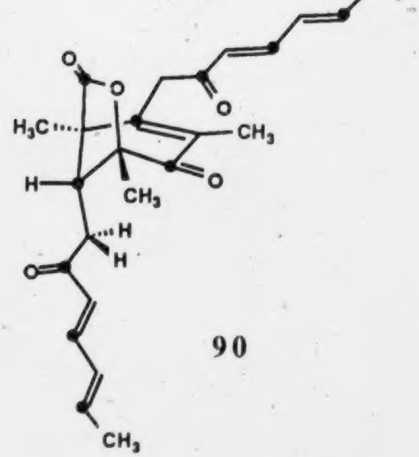
Formation of bislongiquinolide (52) from quinol 75.


O
OH
CH3
H3C
O
H3C
O—H
O
H
H
H
H3C
OH
O
O
CH3
CH3
CH3

88


O
OH
CH3
H3C
H3C
O—H
O
H
H
H
H
CH3
CH3
O
H—O
OH
H3C

89


CH3
O
O
O
H3C
CH3
H
O
CH3
H
H
O
CH3

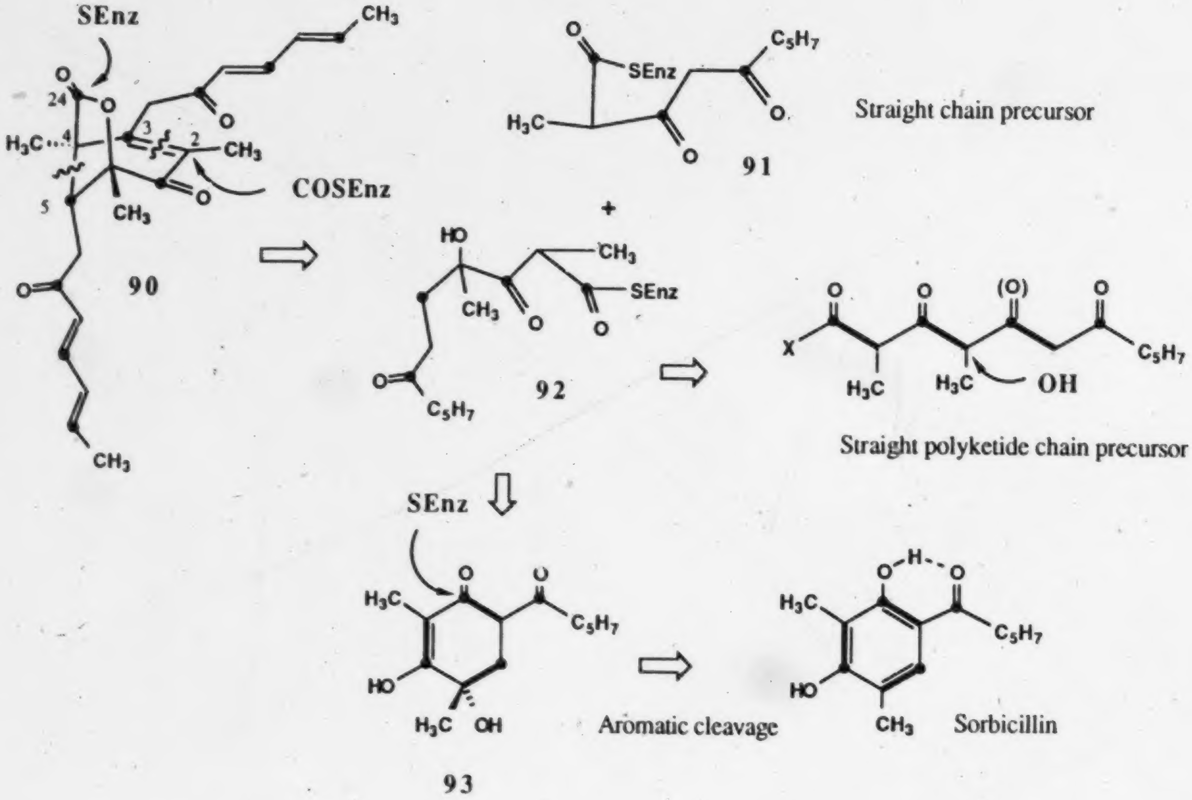
90

Biosynthesis of trichodermolide.

Trichodermolide is an unusual compound in the sense that it is C_{24} compound while the other compounds isolated from *T. longibrachiatum* are either C_{14} or C_{28}. Its biogenesis may involve intermediates which differ from those proposed for the formation of trichodimerol and the other C_{28} compounds. The ^{13}C nmr spectrum of [1-^{13}C]acetate incorporated trichodermolide shows that ^{13}C was incorporated into the expected carbons of the sorbyl chains, and at C-1, C-3, C-5, and C-24, as shown in **90**.

With this infomation in hand we sought to derive a biosynthetic hypothesis for trichodermolide and to devise experiments to probe it. A "retrobiosynthetic" analysis of trichodermolide, as shown in Scheme 6, indicates that trichodermolide could be derived from two different "polyketide" chains. Scission of the C-2, C-3 and C-4, C-5 bonds, followed by opening of the lactone, then addition of a carboxylic end to one of the fragments, provides a methylated pentaketide chain (**91**) and a dimethylated hexaketide chain with a tertiary alcohol (**92**) with the labelling shown in Scheme 6. Direct introduction of a tertiary alcohol in a polyketide chain may not be a plausible mechanism for the formation of **92**. Therefore we suspected that "biosynthon" **92** may arise by a different route.

Interestingly, **92** is also a possible precursor of vertinolide. Lactonization of the tertiary alcohol of **92** with the carboxylic end followed


SEnz
CH3
24
3
2
H3C
CH3
COSEnz
5
CH3
90
CH3
SEnz
C5H7
H3C
91
Straight chain precursor
+
HO
CH3
CH3
SEnz
C5H7
92
X
(O)
C5H7
H3C
H3C
OH
Straight polyketide chain precursor
SEnz
H3C
C5H7
HO
H3C
OH
93
Aromatic cleavage
H3C
C5H7
HO
CH3
Sorbicillin

Scheme 6

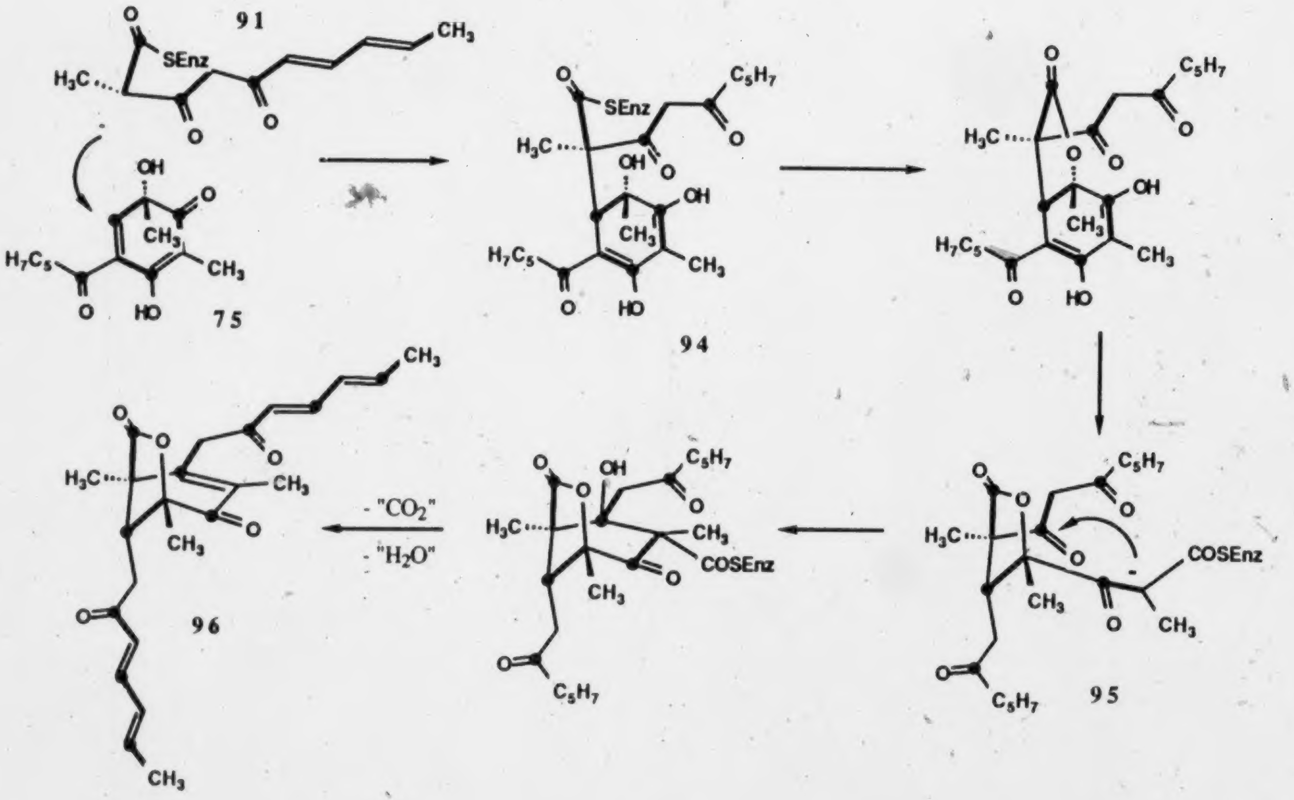
Biogenetic analysis of trichodermolide (**19**).

by tautomerization gives vertinolide. Therefore, the biosynthon **92** may arise from nucleophilic cleavage of an intermediate like **93**, which is a reduced equivalent of **75**. An intermediate such as **75** can be obtained from sorbicillin (**1**) as shown in Scheme 3.

Analysis of the biogenetic hypothesis, shown in Scheme 6, indicated that incorporation of [1,2-$^{13}C_2$]acetate into trichodermolide would provide useful biosynthetic information. As shown in Scheme 6, nucleophilic cleavage of **93** involves splitting an intact acetate unit and the eventual loss of C-1 of acetate. Incorporation of [1,2-$^{13}C_2$]acetate into trichodermolide could demonstrate if such such splitting occurs and hence provide support for our biosynthetic hypothesis. If **92** is a simple polyketide such splitting would not be observed. Unfortunately trichodermolide was not produced on the occasion that we feed sodium [1,2-$^{13}C_2$]acetate to *T. longibrachiatum*.

Therefore at this stage we can only postulate a biosynthesis for the formation of trichodermolide, as shown in Scheme 7. Condensation of the methylated pentaketide **91** with **75** may provide an intermediate (**94**) which can lactonize and cleave to produce an intermediate like **95**. Condensation of the resulting ketoester, followed by hydrolysis of the thioester and loss of CO_2 and water provides trichodermolide with the labelling shown in **96**.

More biosynthetic studies are necessary in order to establish how trichodermolide is produced by *T. longibrachiatum* and these are under way in our laboratory.


91
75
94
95
96
SEnz
COSEnz
- "CO$_2$"
- "H$_2$O"

Scheme 7

Postulated biosynthesis of trichodermolide (**19**).

Table 12. Incorporation of sodium [1-^{13}C]acetate into the metabolites of *Trichoderma longibrachiatum* Rifai aggr.(UAMH 4159).

Compound	Labelled carbons
Trichodimerol (**11**)	57.6 (d), 104.1 (s), 140.3 (d), 143.6 (d), 176.0 (s), 197.9 (s).
Sorbicillin (**1**)	128.2 (d), 140.6 (d), 144.0 (d), 158.2 (s), 162.0 (s), 192.0 (s).
Trichodermolide (**19**)	55.9 (d), 141.5 (d), 142.5 (d), 144.1 (d), 144.7 (d), 149.6 (s), 176.1, 191.2, 195.2, 196.6 (s).
Bisvertinol (**45**)	36.0 (t), 53.6 (d), 106.1 (s), 136.9 (d), 138.9 (d), 140.1 (d), 142.8 (d), 164.4 (s), 168.8 (s), 179.8 (s), 191.6 (s) 191.6 (s).
Bislongiquinolide[1] (**52**)	42.4 (d), 43.7 (d), 140.7 (d), 143.6 (d), 145.2 (d), 147. (d), 169.6 (s), 173.6 (s), 175.6 (s), 195.1 (s), 202.6 (s), 208.2 (s).
Sorbiquinol[2] (**37**)	22.1 (t), 26.1 (t), 46.5 (d), 47.0 (d), 129.0 (d), 130.5 (d 159.0 (s), 162.0 (s), 180.7 (s), 196.9 (s), 202.2 (s), 211.3 (s).

---

[1] The carbons at 42.4 and 43.7 ppm showed a coupling of 33.5 Hz. [2] Isolated as tetrahydrosorbiquinol.

Table 13. Incorporation of sodium [1,2-^{13}C$_2$]acetate into the metabolites of *T.longibrachiatum* Rifai aggr.(UAMH 4159).

Compound	Labelled carbon pairs	Coupling Constant (Hz)
Sorbicillin (**1**)	140.6-18.3	42.7
	144.0-130.0	55.6
	192.0-121.3	57.7
	162.0-113.0	61.3
	158.2-109.9	69.3
	128.2-113.9	63.2
Trichodimerol (**11**)	140.1-18.9	42.5
	57.5-78.7	33.0
	104.0-58.8	38.5
	197.9-102.5	56.4
	175.7-118.3	62.7
	143.0-131.7	56.0

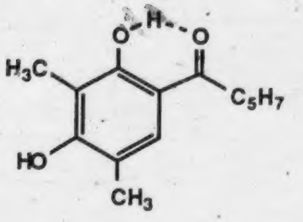
CONCLUSIONS

From the mycelium and culture broth of *Trichoderma longibrachiatum* Rifai aggr. we isolated and determined the structure of the known compounds sorbicillin (**1**), bisvertinol (**45**), and bisvertinolone (**50**), and the new compounds trichodimerol (**11**), trichodermolide (**19**), sorbiquinol (**37**), bislongiquinolide (**52**), and 5-hydroxyvertinolide (**58**).

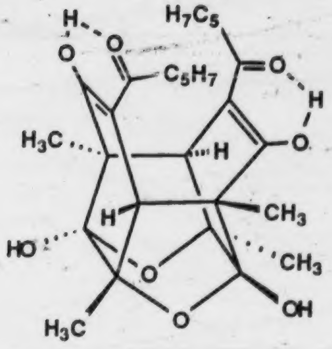
The structure of sorbicillin (**1**) was established by use of a variety of spectroscopic techniques, including nOe experiments and comparison of the results with published data. Furthermore, tetrahydro (**3**) and monoacetate (**73**) derivatives of sorbicillin were prepared, and these confirmed the structure and the ^{13}C nmr assignments.

The structure of trichodimerol (**11**) was determined by spectroscopic techniques, including the use of a [^{1}H]-^{13}C nOe experiment, a natural abundance ^{13}C INADEQUATE experiment, symmetry considerations, and the formation of several derivatives. An octahydro derivative (**12**) was obtained by hydrogenation over palladium on carbon, and several acetylated products of this derivative were obtained: two different asymmetric monoacetates (**13** and **14**), a symmetric diacetate (**15**), an asymmetric triacetate with an endocyclic enol acetate (**16**), and finally a symmetric tetraacetate with endocyclic enol acetates (**18**). The absolute stereochemistry of trichodimerol was determined by use of the exciton chirality method which is non-empirical.

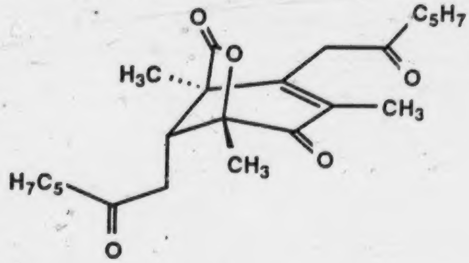
The structure of trichodermolide (**19**) was established using spectroscopic techniques, including nOe and selective INEPT experiments, and chemical transformations. The selective INEPT experiments allowed a


H
O
O
H3C
C5H7
HO
CH3

1

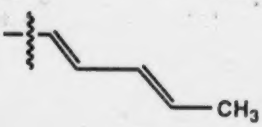

H
O
O
H7C5
C5H7
O
H
O
H3C
H
H
CH3
HO
CH3
O
O
OH
H3C

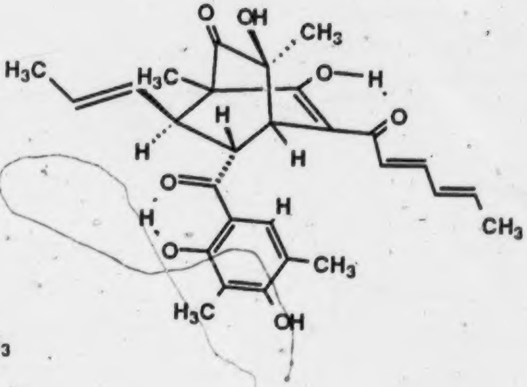
11


O
O
C5H7
H3C
O
CH3
CH3
O
H7C5
O

19

C_5H_7 =

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OH
CH3
H3C
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CH3
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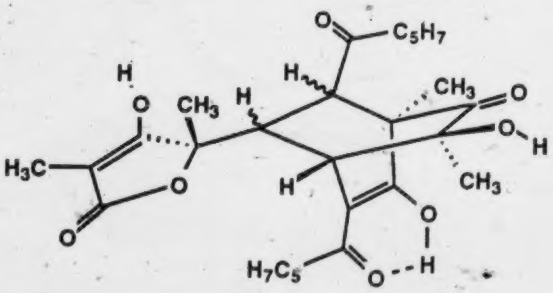
OH

37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CH3
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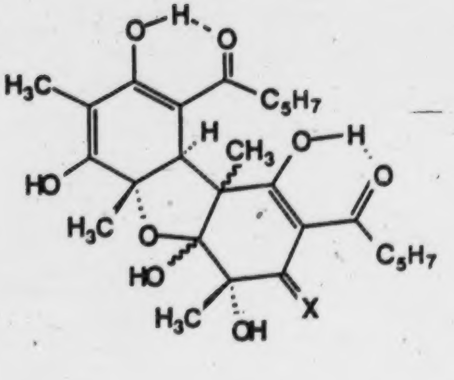
H7C5
O
H

52

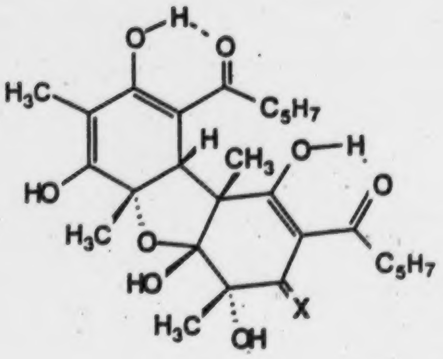
distinction between two possible structures for trichodermolide. Hydrogenation over palladium on carbon gave an octahydro derivative (**25**). Sodium borohydride reduction of this derivative gave two epimeric alcohols (**27a** and **28a**), which gave the corresponding monoacetates (**27b** and **28b**). Treatment of the alcohol mixture with acid provided the anhydro compound **26**, and the aromatic compound **30**. The production of these two derivatives provided chemical evidence for the relative stereochemistry at C-5, and for the carbon skeleton of trichodermolide, respectively. The absolute stereochemistry of trichodermolide was determined by comparison of the CD spectra of the octahydro derivative and compound **34**, whose absolute stereochemistry is known.

The structure of sorbiquinol (**37**) was determined by use of spectroscopic techniques, including extensive nOe experiments. Hydrogenation over palladium on carbon afforded tetrahydro (**43**) and hexahydro (**44**) derivatives. The relative stereochemistry of the asymmetric centers in sorbiquinol was established by nOe experiments, ^{1}H nmr chemical shift considerations, and ^{1}H nmr pyridine solvent shift experiments. The absolute stereochemistry was not determined unambiguously although the CD spectrum displays the characteristic shape of a coupled chromophore system. A tentative assignment is made based on the similarity between sorbiquinol and the other metabolites isolated.

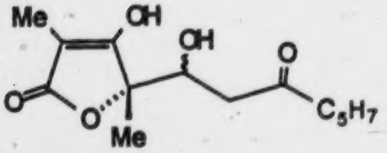
The identity of bisvertinol and bisvertinolone was established by spectropic techniques, including nOe experiments, and comparison of the results with published data. The results of the nOe experiments indicated that the relative stereochemistry of bisvertinol and bisvertinolone, originally assigned by Dreiding and co-workers, should be changed from



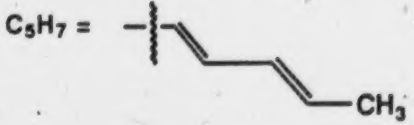
48 $X = H_2$
51 $X = O$



45 $X = H_2$
50 $X = O$



52



that shown in **48** and **51** to that shown in **45** and **50**. Furthermore, the absolute stereochemistry of these compounds was established by use of CD spectroscopy.

The structure of bislongiquinolide is tentatively assigned as **52** with the aid of spectroscopic techniques, including selective INEPT experiments. The relative stereochemistry at C-2 and C-2' is proposed on the basis of ^{1}H nmr chemical shifts and biogenetic considerations. The absolute stereochemistry of this compound requires further study.

The structure of 5-hydroxyvertinolide (**58**) is proposed on the basis of spectroscopic data, ^{1}H nmr coupling constants, and biogenetic considerations. The relative stereochemistry at C-5 is not known, but we tentatively assign the absolute stereochemistry at C-4 on the basis of biosynthetic considerations and its similarity with vertinolide (**49**), the absolute stereochemistry of which is known.

Finally, biosynthetic studies with *T. longibrachiatum* (UAMH 4159) established the polyketide origin of the various compounds isolated and allow us to suggest a possible biosynthetic scheme for most of the metabolites of *T. longibrachiatum*. The biosynthesis of the metabolites was investigated using ^{13}C labelling techniques. Sodium [1-^{13}C]acetate and sodium [1,2-$^{13}C_2$]acetate were administered to growing cultures of *T. longibrachiatum*, the metabolites produced isolated and analyzed by ^{13}C nmr spectroscopy. The results of these experiments are consistent with the formation of trichodimerol, sorbiquinol, bisvertinol, bisvertinolone, bislongiquinolide, and 5-hydroxyvertinolide from sorbicillin, and the formation of sorbicillin from acetate utilizing an unusual folding pattern.

The mode of formation of trichodermolide is unclear. However we are able to suggest a working hypothesis for its formation. Unfortunately, a crucial [1,2-$^{13}C_2$]acetate incorporation experiment failed to provide trichodermolide.

EXPERIMENTAL

General.

High resolution electron impact mass spectra (hreims) were recorded on an AEI MS-50 mass spectrometer coupled to a DS-50 computer. Hreims data are reported as m/z (relative intensity) except for the molecular ion, which is reported as m/z found (m/z calculated, relative intensity). Unless diagnostically significant, peaks with intensity less than 20% of the base peak are omitted. Chemical ionization mass spectra (cims) were recorded on an AEI MS-12 mass spectrometer with ammonia as the reagent gas. The data were processed using DS-55 software and a Nova-4 computer. Cims data is reported as m/z (assignment, relative intensity). Fourier transform infrared (ftir) spectra were recorded (as a $CHCl_3$ cast unless otherwise noted) on a Nicolet FTIR 7199 interferometer. Ultraviolet (uv) spectra were recorded on a Hewlett Packard 8450A diode array spectrophotometer. Optical rotations were recorded on a Perkin Elmer 141 polarimeter. ^1H and ^{13}C nuclear magnetic resonance (nmr) spectra were measured (in $CDCl_3$ unless noted) on Bruker WM-360 and WH-300 (operating at 75.5 MHz ^{13}C nmr) spectrometers, coupled to Aspect 2000 and Aspect 3000 computer systems, respectively. Chemical shifts are reported in parts per million (δ value from tetramethylsilane (TMS)). The residual solvent signal was used as the internal standard, $CDCl_3$: ^1H: δ 7.262 ppm; ^{13}C: δ 77.00 ppm; acetone-d_6: ^1H: δ 2.04 ppm, ^{13}C: δ 29.0 ppm; MeOH-d_4: ^1H: δ 3.30 (methyl) ppm, ^{13}C: δ 49.00 ppm; C_6D_6: δ ^1H: 7.15 ppm, ^{13}C: δ 128.00

ppm relative to TMS. Coupling constants, *J*, are expressed in hertz (Hz) and the following abbreviations are used: m = multiplet, s = singlet, d = doublet, t = triplet, q = quartet, br = broad. Melting points were measured on a Zeitz-Wetzlar or a Thomas model 40 melting point apparatus and are uncorrected.

Reagent grade solvents were distilled prior to use. Skellysolve B refers to Skelly oil company light petroleum, Bp 62-70 C. Analytical grade diethyl ether (ACS 288) was used without further purification.

Pharmacia Sephadex LH-20 was used for the Sephadex columns. Fractions were collected with an ISCO model 820 fraction collector. Medium pressure silica gel column (flash) chromatography was carried out with Merck Silica Gel 60, 230-400 mesh ASTM, according to the method described by Still (94). Analytical thin-layer chromatography (tlc) was carried out on silica gel precoated aluminium tlc plates (E. Merck DC-Alufolien, Kieselgel 60 F-254 0.2 mm thickness). The chromatograms were examined under ultraviolet light (254 nm). Visualization was completed by dipping into a 3% phosphomolybdic acid solution (10 g of MoO_3 H_3PO_4, 1.25 g of $Ce(SO_4)_2$, 12 mL concentrated H_2SO_4, diluted to 250 mL) and charing on a hot plate (300 C).

Culturing of the fungi and extraction and isolation of the metabolites.

Source of the fungus.

T. longibrachiatum (strain UAMH 4159) was obtained from the University of Alberta Microfungus Collection and Herbarium. It was originally deposited as *Trichoderma reesei* Simmons, mutant of ATCC 24449 (QM 9123). This strain (UAMH 4159) has previously been deposited at the American Type Culture Collection (ATCC) as ATCC 26921, at the Commonwealth Mycological Institute (CMI) as CMI 192656, and at the Quartermaster Research and Development Center (QM) as QM 9414. *T. reesei* ATCC 24449 is a mutant of *T. reesei* ATCC 13631 (*T. viride* QM 6a) (CMI 45548), which was isolated from a "cotton duck shelter" by D.I. Fennell (95).

T. viride QM 6a has been used for its production of cellulase (96) and it is known to produce cell-wall lytic enzymes (97). Several mutants and mutants of mutants of *T. viride* QM 6a (e.g. ATCC strains 28217, 24449, 26920, 26921, 28217, 46480, 46481, 58350, 58351, 58352, and 58353) have been found to be good producers of cellulases.

T. longibrachiatum (UAMH 5068) was obtained from Professor Edgar Vargas of the University of Costa Rica and deposited at the University of Alberta Microfungus Collection and Herbarium by Lois Browne. Professor Vargas obtained it from the National University of Costa Rica (98). However, details of its origin are not available.

Inoculum Preparation.

Slant cultures (PDA) were stored in a refrigerator at 4°C. A small piece of mycelium and agar was removed from the slant culture and was used to inoculate an agar plate (PDA), and the plate culture was allowed to grow for one week under partial (12 hours/day) light exposure and room temperature. A portion of the plate culture was used to inoculate a liquid culture (potato dextrose media, 250 mL) and was allowed to grow with constant shaking (160 r.p.m.) for 1 week. Inocula (20 mL) from the small shake culture was used to inoculate still cultures grown in Fernbach flasks (1 L, media composition: *vide infra*), while inocula (25 mL) was used to inoculate large shake cultures (500 mL of media).

Culture conditions.

T. longibrachiatum strain UAMH 5068 was grown in still and shake culture at room temperature under partial light exposure (12 hours/day) using a medium containing: glucose 2%, yeast extract 0.5%, malt extract 0.5%, K_2HPO_4 0.25%, $MgSO_4$ 0.2%, $(NH_4)_2SO_4$ 0.1%, bactopeptone 0.5%. The still culture was harvested after 26 days and the shake culture after 11 days of growth.

T. longibrachiatum strain UAMH 4159 was grown on still culture using the same media, but with lactose instead of glucose as the main carbon source. This growth was harvested after 27 days.

Extraction of the Metabolites.

The mycelium was separated from the broth by gravity filtration through cheese cloth. The mycelial mass was dried for 24 hours in a fume hood and then extracted for 24 hours with chloroform/dichloromethane 1:1 in a percolator. The solvent was removed under reduced pressure to give the crude mycelial extract.

The broth was concentrated under reduced pressure to approximately 1/4 of its original volume. Butanol was added to avoid excessive foaming during the evaporation. The concentrated broth was then extracted under stirring with chloroform (approx. 4 x 1/2 volume). The chloroform extract was concentrated under reduced pressure to give the crude broth extract as a gum. The yield obtained of mycelia and broth extracts from different growths is shown on Table 14.

Isolation of the metabolites.

The crude mycelium extract (9.5g) from a still culture of the *T. longibrachiatum* (UAMH 5068) was separated in the following way: Sephadex LH-20 chromatography (column: 70g, 36 x 2.8 cm i.d.); the column was packed in methanol, and increasing amounts of chloroform and Skellysolve B were used to precondition the column and the column was rinsed with 250 mL of the least polar solvent mixture to be used. Elution of the metabolites from the crude extract was accomplished using a stepwise increase in solvent polarity. The solvents used were Skellysolve B:$CHCl_3$ 2:3 (500 mL), 1:2 (750 mL), Skellysolve B:$CHCl_3$:acetone 1:2:1

(250 mL), 1:2:2 (250 mL), $CHCl_3$:acetone 1:1 (250 mL), methanol. The first fraction (75 mL) was discarded and subsequent fractions (15-20 mL) collected and like fractions (tlc) combined.

Table 14. Metabolite production by *Trichoderma longibrachiatum* Rifai aggr. (UAMH 5068 and UAMH 4159) under different culture conditions.

Culture	Mycelial weight (gL^{-1})	Mycelial extract (gL^{-1})	Broth extract (gL^{-1})	Growth time (days)
Trichoderma longibrachiatum (UAMH 5068):				
Fermentor	7.5	0.07	0.32	4
Shake	8.0	0.25	0.74	11
Still	3.1	0.95	0.75	26
Trichoderma longibrachiatum (UAMH 4159):				
Still	4.4	0.93	1.7	27
Feeding experiments:				
Still (1-^{13}C)	5.6	0.25	0.42	22
Still (1,2-$^{13}C_2$)	4.8	0.26	-	26

Pure trichodimerol eluted in fractions 20-33 and 56-73 (0.86 g). Sorbicillin eluted in fractions 34-44 as a 1:1 mixture with trichodimerol (0.26g). Sorbiquinol coeluted with trichodimerol in fractions 45-55 (0.27g). Crude bisvertinol eluted in fractions 74-82 (3.5 g). Bisvertinol was purified further by column chromatography (Sephadex LH-20, Skellysolve B/$CHCl_3$/Acetone 1:1:0.4) to give 470 mg of bisvertinol. An analytical sample, prepared by precipitation (twice) from Skellysolve B/Et_2O gave high purity bisvertinol (82 mg). Column chromatography (silica gel; 1% EtOH/$CHCl_3$) of the fraction containing sorbicillin (60 mg) gave pure sorbicillin (20 mg). Column chromatography (silica gel; 2% EtOH/$CHCl_3$) of the fraction containing sorbiquinol (80 mg) afforded sorbiquinol (19.4 mg) and after increasing the polarity (6% EtOH/$CHCl_3$) trichodimerol (19.4 mg).

The broth extract (7.5 g) of the still culture of *T. longibrachiatum* (UAMH 5068) was separated as described above. Fractions 15-30 contained impure trichodimerol (0.76 g). Fractions 31-35 gave sorbicillin and trichodimerol (87 mg), 36-57 impure trichodimerol (0.6 g), and 58-69 impure bisvertinol (1.70 g). Sorbiquinol was not obtained. After repeating the Sephadex column with fractions 4-15 (3.25 g), 370 mg of bislongiquinolide was obtained in fraction 79-85 and 100 mg of bisvertinolone in fractions 87-90.

The crude broth extract (3.7 g) from the shake culture of *T. longibrachiatum* (UAMH 5068) was separated as before by Sephadex LH-20 chromatography. Impure bisvertinolone was obtained in fractions 56-65. This was precipitated from Skellysolve B/Et_2O to give 350 mg of bisvertinolone as an amorphous powder.

The mycelium extract of *T. longibrachiatum* (UAMH 4159) (9.3 g) was separated by Sephadex chromatography as described above. Trichodermolide was found in fractions 1-3 and was purified by column chromatography (Si gel, Bz/Et_2O 10:1 to 5:1) to give 235 mg of pure trichodermolide. Fractions 11-18 contained almost pure trichodimerol (1.5 g), fractions 19-38 gave mainly a mixture of trichodimerol, sorbicillin, and sorbiquinol (2.4 g), fractions 58-66 impure bisvertinol (1.5 g).

The broth extract of *T. longibrachiatum* (UAMH 4159) (8 g) was separated by Sephadex chromatography as described above. Fractions 1-5 contained impure trichodermolide, which after column chromatography (Si gel Bz:Et_2O 3:1) gave 71 mg of pure trichodermolide. Fractions 32-50 (1.2 g) were combined and subjected to further Sephadex chromatography using Skellysolve B:$CHCl_3$:acetone (2:3:0.4) as eluant. The first 150 mL were discarded. 5-Hydroxyvertinolide eluted in fractions 34-48, with fractions 39-40 containing pure 5-hydroxyvertinolide (40 mg).

A summary of the metabolites isolated from the different extracts of *T. longibrachiatum* is shown on Table 15.

Table 15. Metabolites present in the extracts of *Trichoderma longibrachiatum* Rifai aggr.[1]

	Extract						
	Fermentor	Shake culture		Still culture (UAMH 5068)		Still culture (UAMH 4159)	
Compound	Broth	Broth	Mycelium	Broth	Mycelium	Broth	Mycelium
Sorbicillin	-	-	?	+	++	-	+
Bisvertinol	?	+		++	++	++	++
Bisvertinolone	++	++		++	-		-
Trichodimerol	+	-	++	++	++	+	++
Sorbiquinol	-	-	-	?	++	-	+
Trichodermolide	?		?	?	+	++	++
Hydroxyvertinolide	?			?	?	+	-
Bislongiquinolide	++	-		++	-	+	-

[1] The presence of a compound was determined by a combination of tlc, purification, and ^1H nmr spectroscopy of the respective fractions. A question mark indicates the presence of the compound was indicated by tlc only.

Spectral characterization of the individual compounds.

Sorbicillin (**1**).

ftir υ_{max} (cm^{-1}): 3360, 2920, 1640, 1620, 1559, 1480, 1383, 1281, 1154 and 990; uv (MeOH) λ_{max} nm (ε): 318 (27000); NaOH: 405 (29000) HCl regenerated the original spectrum; ^{1}H nmr spectrum: δ 1.92 (d, 6 Hz 3H), 2.16 (s, 3H), 2.24 (s, 3H), 5.26 (br. s, 1H), 6.32 (dq, 12, 6 Hz, 1H) 6.38 (dd, 10, 12 Hz, 1H), 6.98 (d, 15 Hz, 1H), 7.48 (dd, 10, 15 Hz, 1H) 7.48 (s, 1H), 13.58 (s, 1H); (C_6D_6, 200 MHz): δ 1.44 (d, 6.5 Hz, 1H), 1.94 (s, 3H), 1.96 (s, 3H), 4.62 (br.s, 1H, exhanges upon addition of D_2O), 5.70 (dq, 15, 6.5 Hz, 1H), 6.00 (ddq, 11, 15, 1.5 Hz, 1H), 6.73 (d, 14.5 Hz, 1H) 7.24 (s, 1H), 7.61 (dd, 10.5, 14.5 Hz, 1H), 14.42 (s, 1H); ^{13}C nm spectrum: δ 7.0 (q), 15.1 (q), 18.4 (q), 109.9 (s), 113.0 (s), 113.9 (s), 121.3 (d), 128.2 (d), 130.0 (d), 140.6 (d), 144.0 (d), 158.2 (s), 162.0 (s), 192.0 (s); hreims: 232.1096 (calcd for $C_{14}H_{16}O_3$: 232.1100, 97%), 217 (100) 191 (70), 189 (20), 175 (25), 165 (68), 136 (72), 95 (6); cims: 233 (M+1 100); tlc: 0.37-0.41 (2% EtOH/$CHCl_3$), 0.61-0.63 (6%).

Trichodimerol (**11**).

$[\alpha]_D$ -376° (*c* 0.26, MeOH); ftir υ_{max} (cm^{-1}): 3420, 2970, 2920, 1613 1546, 1414, 1288, 1251, 1150, 1125, 992, 935; uv (MeOH) λ_{max} nm (log ε): 362 (4.48), 307, (4.20), 295 (4.17), 240 (4.00); NaOH: 378 nm; HCl regenerated the original spectrum; CD (MeOH) $\lambda_{extremum}$ nm (Δε): 336 (+11), 382 (-14); amplitude (*A*) = -25; ORD (MeOH) $\lambda_{extremum}$ nm ([ϕ]): 230 (+10900), 296 (-22800), 357 (+52600), 408 (-20000); ^{1}H nm spectrum: see Table 1; ^{1}H nmr spectrum (C_6D_6, 360 MHz): δ 1.42 (dd, 1.6, 7 Hz, 1H), 1.46 (s, 3H), 1.54 (s, 3H), 3.12 (s, 1H), 3.35 (br. s, 1H), 5.55 (dq, 15, 7 Hz, 1H), 5.88 (ddq, 15, 11, 1.5 Hz, 1H), 6.25 (d, 15 Hz, 1H), 7.38 (dd, 11, 14.5 Hz, 1H), 17.14 (s, 1H); ^{13}C nmr spectrum ($CDCl_3$): see Table 2; (C_6D_6): ; hreims: 496.2090 (calcd for $C_{28}H_{32}O_8$: 496.2098, 14%), 401 (3), 248 (22), 232 (7), 205 (30), 137 (33), 95 (C_6H_7O, 100); cims: 514 (M+18, 0.4%), 497 (M+1, 1.2%), 249 (1/2 M+1, 30%); fabms: 497 (M+1, 35%), 249 (M/2+1, 30%); tlc: 0.73 (CH_2Cl_2/acetone 3:1).

Trichodermolide (**19**).

$[\alpha]_D$ +97°(*c* 0.98, EtOH); ftir υ_{max} (cm^{-1}): 3015, 2920, 1781, 1687, 1637, 1595, 1375, 1320, 1188, 997; uv (MeOH) λ_{max} nm (log ε): 280 (4.72), 217 (4.15); no change upon addition of NaOH or HCl; CD (EtOH) $\lambda_{extremum}$ nm (Δε): 224 (-15.4), 260 (+5.4), 273 (+2.9), 289 (+9); ORD (EtOH) $\lambda_{extremum}$ nm ([ϕ]): 240 (-49000), 278 (-30000), 300 (+15000); ^1H nmr spectrum: see Table 5; ^{13}C nmr spectrum: see Table 6; hreims: 396.1936 (calcd for $C_{24}H_{28}O_4$: 396.1937, 0.7%), 352 ($C_{23}H_{28}O_3$, 0.2%), 340 (0.2), 301 (0.2), 258 (0.3), 257 (0.7), 248 (1), 191 (2.8), 95 (100); cims: 414 (M+18, 15%), 397 (M+1, 100%); tlc: 0.45 (Skellysolve B/EtOAc 5:2).

Sorbiquinol (**37**).

$[\alpha]_D$ +234° (*c* 0.36, MeOH); ftir υ_{max} (cm^{-1}): 3450, 3020, 2960, 2920, 2850, 1730, 1627, 1606, 1562, 1380, 1166, 995; uv (MeOH): see Table 11; CD (MeOH) $\lambda_{extremum}$ nm (Δε): 343 (+25), 296 (-28); *A* = +53; ORD (MeOH) $\lambda_{extremum}$ nm ([ϕ]): 362 (+38000), 323 (-85000), 272

(+73000), 252 (+85000); ^{1}H nmr spectrum: see Table 8; (acetone-d_6): δ 1.07 (s, 3H), 1.20 (s, 3H), 1.58 (dd, 7, 1.5 Hz, 3H), 2.24 (s, 3H), 3.19 (dd, 6.5, 10 Hz, 1H), 3.50 (d, 1.5 Hz, 1H), 3.79 (br. s, 1H), 4.48 (dd, 6.5 Hz, 1.5 Hz, 1H), 5.16 (ddq, 15, 10, 1.5 Hz, 1H), 5.45 (dq, 15, 7 Hz, 1H), 5.82 (d, 15 Hz, 1H), 5.95 (ddq, 15, 10, 1.5 Hz, 1H), 6.13 (dq, 15, 7 Hz, 1H), 7.12 (dd, 10, 15 Hz, 1H), 7.69 (s, 1H), and 8.05 (br. s, 1H) ppm; ^{13}C nmr spectrum: see Table 9; hreims: 480.2132 (calcd for $C_{28}H_{32}O_7$: 480.2149, 17%), 452 (2), 434 (1), 248 (2), 233 (18), 232 (32), 217 (22), 191 (8), 165 (100), 95 (31); cims: 481 (M+1, 12%), 249 (24), 233 (100); tlc: 0.26-0.29 (2% EtOH/$CHCl_3$), 0.48-0.51 (6%).

Bisvertinol (**45**).

mp 151.5-152.5°C (powder from Skellysolve B/Et_2O); $[\alpha]_D$ -1274° (*c* 0.99, MeOH); ftir υ_{max} (cm^{-1}): 3400, 3010, 2975, 2925, 1616, 1556, 1445, 1411, 1379, 1022, 992, 974; uv (MeOH) λ_{max} nm (ε): 227 (9900), 274 (15400), 300 (15000), 313sh, 400 (24000); NaOH: 203 (74000), 234 (14200), 273 (17300), 298 (14700), 362 (18000), 426 (17000); HCl regenerated the original spectrum; CD (MeOH) $\lambda_{extremum}$ nm (Δε): 410 (-42), 353 (+33); *A* = -75; ORD (MeOH) $\lambda_{extremum}$ nm ([ϕ]): 443 (-53000), 379 (+227000), 315 (+20000), 276 (+31000); ^{1}H nmr spectrum (MeOH-d_4, 360 MHz): δ 1.19 (s, 3H), 1.26 (s, 3H), 1.40 (s, 3H), 1.42 (s, 3H), 1.85 (d, 7 Hz, 3H), 1.89 (d, 7 Hz, 3H), 2.44 (d, 14 Hz, 1H), 2.71 (d, 14 Hz, 1H), 3.63 (s, 1H), 6.09 (ddq, 10, 15, 1.5 Hz, 1H), 6.25-6.40 (m, 3H), 7.19 (dd, 10, 15, 1H), 7.20 (dd, 10, 15 Hz, 1H); ^{1}H nmr spectrum: δ 1.30 (s, 3H), 1.38 (s, 3H), 1.50 (s, 3H), 1.58 (s, 3H), 1.92 (d, 6 Hz, 6H), 2.62 (d, 14 Hz, 1H), 2.75 (d, 14 Hz, 1H), 3.68 (s, 1H), 3.98 (br. s, 1H), 6.4

(m, 6H), 6.44 (d, 15 Hz, 1H), 7.34 (dd, 15, 11 Hz, 2H), 16.2 (s, 1H), 16.7 (s, 1H); ^{13}C nmr spectrum (MeOH-d_4, 75.5 MHz): δ 7.1 (q), 18.8 (q), 18.9 (q), 20.0 (q), 22.7 (q), 25.8 (q), 36.5 (t), 54.9 (d), 60.3 (s), 74.2 (s), 80.4 (s), 102.5 (s), 106.0 (s), 107.2 (s), 110.6 (d), 121.7 (d) 2C, 132.2 (d), 137.2 (d), 139.3 (d), 140.5 (d), 143.3 (d), 168.4 (s), 170 (s), 179.7 (s), 193.3 (s), 194 (s); (all carbons within 0.4 ppm of reported values); hreims: 250.1206 (calcd for $C_{14}H_{18}O_4$: 250.1205, 11%), 248 ($C_{14}H_{16}O_4$, 8%), 233 (16), 232 (100), 231 (21), 218 (13), 217 (84), 191 (34), 189 (19), 165 (41), 136 (25), 95 (100); cims: 498 (M, 7%), 249 (82%), 233 (96%); tlc: 0.08-0.13 (2% EtOH/$CHCl_3$), 0.31-0.37 (6%).

Bisvertinolone (**50**).

mp 153-155°C (diisopropylether), 124-5° C Skellysolve B/Et_2O); ftir ν_{max} (cm^{-1}): 3400, 3020, 2980, 2935, 1760w, 1663s, 1607, 1575, 1412, 1379, and 994; uv (MeOH): 272 (23000), 294 (sh (23000), 366 (24000); NaOH: 276 (32000), 394 (17000); HCl regenerated the original spectrum; CD (MeOH) $\lambda_{extremum}$ nm ($\Delta\varepsilon$): 402 (-22), 350 (+20); *A* = -42; ORD (MeOH) $\lambda_{extremum}$ nm ([ϕ]): 437 (-23500), 370 (+91000), 325 (+12000); ^{1}H nmr spectrum: δ 1.35 (s, 3H), 1.42 (s, 3H), 1.43 (s, 3H), 1.46 (s, 3H), 1.86 (d, 6 Hz, 1H), 1.89 (d, 5 Hz, 1H), 3.72 (s, 1H), 4.20 (br. s, 1H, exchangeable), 4.54 (br. s, 1H, exchangeable), 6.0-6.4 (m, 6H, 1H exchangeable), 7.31 (dd, 11, 15 Hz, 1H), 7.39 (d, 15 Hz, 1H), 7.56 (dd, 16, 10 Hz, 1H), 16.31 (s, 1H), 17.71 (s, 1H); ^{13}C nmr spectrum: δ 6.9 (q), 18.6 (q), 18.7 (q), 19.2 (q), 22.6 (q), 25.6 (q), 54.4 (d), 59.8 (s), 79.0 (s), 79.8 (s), 99.8 (s), 104.0 (s), 107.2 (s), 110.9 (s), 120.0 (d), 121.9 (d), 131.0 (d), 131.2 (d), 137.4 (d), 139.6 (d), 144.0 (d), 148.3 (d), 163.8 (s), 170.2

(s), 185.7 (s), 191.2 (s), 196.4 (s), 199.8 (s); hreims: 264.0996 (calcd for $C_{14}H_{16}O_5$: 264.0998, 38%), 248 ($C_{14}H_{16}O_4$, 15%), 242 (7), 222 (17), 221 (84), 180 (22), 153 (93), 95 (100); cims: 266 (4), 249 (37), 222 (17); tlc: 0.10-0.17 (2% EtOH/$CHCl_3$), 0.33-0.37 (6%); nOe (C_6D_6): [1.19]: 1.56 (0.8%), OH approx. 6 (1.4%); [1.407]: 4.01 (0.4%), OH approx. 6 ppm (3.5%); [1.56]: 4.01 (0.5%), OH (4.4%), 6.12 (d, 15 Hz) (1%); [1.97]: OH (7.5%); [4.01]: 6.12 approx. (d, 15 Hz) (6.6%), 1.56 (2.6%), 1.407 (3.5%) ppm. nOe ($CDCl_3$): [3.72]: 6.38 (8%), 1.42-3 (7%); [1.46]: none; [1.42-3]: 3.72 (3.6%); [1.35]: none.

Bislongiquinolide (**52**).

$[\alpha]_D$ +105° (*c* 0.55, MeOH); ftir υ_{max} (cm^{-1}): 3400, 3050, 2980, 2920, 1732, 1660, 1626, 1602, 1563, 1380, 1065, 998. uv (MeOH) λ_{max} nm (ε): 262 (19000), 292 (20000), 370 (15000), 385sh; NaOH: 204 (83000), 259 (21000), 287 (22000), 393 (11000); HCl: 232 (11000), 294 (18000), 368 (17000), 385sh; CD (MeOH) $\lambda_{extremum}$ nm ($\Delta\varepsilon$): 360 (+11), 320 (-18), 281 (7), 230 (-8); ORD (MeOH) $\lambda_{extremum}$ nm ([ϕ]): 390 (+26000), 338 (-64000), 300 (+67000), 267 (+4500); ^1H nmr spectrum: δ 1.10 (s, 3H), 1.28 (s, 3H), 1.49 (s, 3H), 1.56 (s, 3H), 1.90 (d, 7 Hz, 3H), 1.92 (d, 7 Hz, 3H), 3.18 (dd, 2, 6 Hz, 1H), 3.34 (d, 2 Hz, 1H), 3.39 (d, 6 Hz, 1H), 5.00 (br. s, 1H), 6.12 (d, 15 Hz, 1H), 6.16 (d, 15 Hz, 1H), 6.2-6.45 (m, 5H), 7.23 (dd, 10.5, 15 Hz, 1H), 7.34 (dd, 10.5, 15 Hz, 1H), 14.02 (br. s, 1H); ^{13}C nmr spectrum: δ 6.3 (q), 11.1 (q), 19.0 (q), 19.2 (q), 23.2 (q), 23.5 (q), 42.4 (d), 43.6 (d), 51.3 (d), 62.6 (s), 74.7 (s), 82.9 (s), 98.2 (s), 108.5 (s), 117.6 (d), 127.0 (d), 130.2 (d), 130.9 (d), 140.6 (d), 143.8 (d), 145.4 (d), 147.7 (d), 169.7 (s), 174.2 (s), 176.1 (s), 194.9 (s), 202.6 (s), 208.2 (s); hreims

(70 eV): 496.2089 (calcd for $C_{28}H_{32}O_8$: 496.2098, 5%), 478 (2), 468 (2), 373 (21), 95 (100), 67 (30). lreims (16eV): 496 (52), 453 (22), 373 (55), 248 (100), 232 (70), 217 (30), 180 (62), 95 (93).

5-Hydroxyvertinolide (**58**).

$[\alpha]_D$ -64°(*c* 0.11, MeOH); ftir υ_{max} (cm^{-1}): 3360, 3025, 2987, 2965, 2936, 1749, 1729, 1666, 1638, 1593, 1447, 1402, 1306, 1064, 1020, 762; uv (MeOH) λ_{max} nm (ε): 230sh, 263 (13000), 359 (900); NaOH: 203 (19000), 260 (16000), 373 (700); HCl: 229 (8100), 274 (10400), 357 (1100); ^1H nmr spectrum (acetone-d_6, 360 MHz): δ 1.49 (s, 3H), 1.71 (s, 3H), 1.90 (d, 6.5 Hz, 3H), 2.77 (dd, 16, 10 Hz, 1H), 3.15 (dd, 16, 2 Hz, 1H), 3.49 (s, 1H), 4.14 (dd, 2, 10 Hz, 1H), 6.08 (d, 15 Hz, 1H), 6.1-6.4 (m, 2H), 7.21 (dd, 16, 10 Hz, 1H), 10 (v. br. s., 1H); ^{13}C nmr spectrum (acetone-d_6, 75.5 MHz): δ 5.3 (q), 18.1 (q), 18.1 (q), 40.52 (t), 70.3 (d), 82.8 (s), 96.4 (s), 127.2 (d), 129.7 (d), 140.6 (d), 143.5 (d), 173.4 (s), 175.4 (s), 198.5 (s).

Preparation of Derivatives.

Hydrogenation of sorbicillin (1).

A fraction containing sorbicillin (200 mg), obtained from the mycelial extract of *T. longibrachiatum* (UAMH 5068), was hydrogenated over excess palladium on carbon, in ethyl acetate (10 mL), at room temperature and pressure, overnight. The reaction mixture was filtered, concentrated, and purified by column chromatography (silica gel, 2% EtOH/$CHCl_3$) to give tetrahydrosorbicillin (**3**) (80 mg, approx. 80% yield).

Hydrogenation of trichodimerol (**11**).

Trichodimerol (**11**) (170 mg) in ethyl acetate was hydrogenated over palladium on carbon at room temperature for one hour. The reaction mixture was filtered, concentrated, and purified by column chromatography (silica gel Bz:Et2O 1:1) to give octahydrotrichodimerol (**12**) (105 mg, 61% yield).

Acetylation of tetrahydrosorbicillin (**3**).

Tetrahydrosorbicillin (**3**) (10 mg) was acetylated by reaction with acetic anhydride (0.5 mL) and pyridine (0.25 mL) at room temperature (2 hr.). The excess pyridine and acetic anhydride was evaporated azeotropically with toluene to give pure O-acetyl-tetrahydrosorbicillin (**73**) (10 mg).

Acetylation of octahydrotrichodimerol (**12**).

Octahydrotrichodimerol (129 mg), acetic anhydride (1.5 mL), pyridine (0.5 mL), and dimethylaminopyridine (one crystal) were refluxed in dichloromethane (15 mL) for seven days. Methanol (5 mL) was added, the reaction mixture filtered, and evaporated in *vacuo*. Traces of pyridine were removed by azeotropic evaporation with toluene. Purification by column chromatography (silica gel; 12 to 33% ethyl acetate:Skellysolve B) afforded monoacetyloctahydrotrichodimerol (**13**) (3.1 mg), diacetyloctahydrotrichodimerol (**15**) (51.2 mg), triacetyloctahydrotrichodimerol (**16**) (32.3 mg), and impure monoacetyloctahydrotrichodimerol (**14**) (7.7 mg). Compound **14** was purified further by preparative tlc on silica gel (eluant: 4% EtOH/$CHCl_3$) to give pure monoacetyloctahydrotrichodimerol (**14**) (3 mg).

Hydrogenation of trichodermolide (**19**).

Trichodermolide (58 mg) in ethyl acetate (10 mL) was hydrogenated over excess palladium on carbon for one hour. The reaction mixture was filtered, concentrated, and purified by column chromatography (silica gel Bz:Et_2O 3:1, Rf 0.74) to give pure octahydrotrichodermolide (**21**) (30.1 mg, 51% yield).

Reduction of octahydrotrichodermolide (**25**).

Octahydrotrichodermolide (**25**) (22 mg) was dissolved in benzene (2 mL), cooled to 0°C, and excess sodium borohydride in ethanol was added at 0°C. The reaction was quenched after 1.5 minutes by addition of saturated aqueous ammonium chloride (0.5 mL). The reaction mixture was diluted with water (8 mL), and extracted with ethyl acetate (3x15 mL). The ethyl acetate extract was concentrated and purified by column chromatography (silica gel, 2% EtOH/$CHCl_3$ Rf = 0.24) to give α-decahydrotrichodermolide (**27a**) (10.0 mg, 45% yield) and traces of starting material.

Octahydrotrichodermolide (**25**) (21.5 mg) was dissolved in benzene (1 mL), and sodium borohydride (14.1 mg) in ethanol (1 mL) was added at 0°C. After two minutes the reaction was quenched by addition of saturated aqueous ammonium chloride (0.5 mL). The reaction mixture was diluted with water (5 mL) and extracted with chloroform (3x5 mL). The chloroform extract was evaporated, and the crude mixture was treated with p-toluensulphonic acid (10 mg) in methanol (4 mL) for two weeks. The methanol was concentrated and the mixture purified by column chromatography (silica gel; 2% EtOH/$CHCl_3$) to afford β-decahydrotrichodermolide (2.8 mg) (**28a**). Use of 3% Et_2O/Bz as eluant afforded aromatic compound **30** (2.6 mg) and the enol ether anhydrodecahydrotrichodermolide (**26**) (2.4 mg).

α-Decahydrotrichodermolide (**27a**) (4.5 mg) was treated with 1% methanolic p-toluensulphonic acid (2 mL), at room temperature for two hours. The reaction mixture was concetrated and purified by column chromatography (silica gel; 2% EtOH/$CHCl_3$), to give the enol ether anhydrodecahydrotrichodermolide (**26**) (3.5 mg, Rf = 0.71).

Acetylation of α-decahydrotrichodermolide (**27a**).

α-Decahydrotrichodermolide (**27a**) (3 mg) was acetylated by overnight reaction with acetic anhydride (1 mL) and pyridine (0.5 mL) at room temperature. The excess pyridine and acetic anhydride was evaporated azeotropically with toluene to give pure O-acetyl–α-decahydrotrichodermolide (**27b**) (2.5 mg). Acetylation of β-decahydrotrichodermolide (**28a**) in a similar way gave O-acetyl-β-decahydrotrichodermolide (**28b**) (1.5 mg).

Aromatization of anhydrodecahydrotrichodermolide (**26**).

Anhydrodecahydrotrichodermolide (**26**) (8 mg) was refluxed overnight in 1% methanolic p-toluensulphonic acid (2 mL). The reaction mixture was evaporated and purified by column chromatography (3% Et_2O/Bz) to give the aromatic compound **30** (4 mg) and unreacted starting material.

Hydrogenation of sorbiquinol (**37**).

A mixture (58 mg) containing sorbiquinol and trichodimerol, obtained from the mycelial extract of *T. longibrachiatum* (UAMH 5068), was hydrogenated with excess palladium on carbon in ethyl acetate (5 mL) at room temperature and pressure for one hour. The reaction mixture was filtered (though celite), evaporated, and purified by column chromatography (silica gel, 3% EtOH/$CHCl_3$) to give tetrahydrosorbiquinol (**43**) (15 mg, 95% pure) and octahydrotrichodimerol (**12**) (20 mg).

When the hydrogenation is allowed to proceed for two days, work up of the reaction mixture in a similar way gave hexahydrosorbiquinol (**44**) (6 mg).

Spectral data of the derivatives.

Tetrahydrosorbicillin (**3**).

ftir υ_{max} (cm^{-1}): 3450, 2955, 2920, 2850, 2830, 1630, 1592, 1480, 1161, 1110; uv (MeOH) λ_{max} nm (ε): 214 (145000), 229sh, 284 (10400), 327 (4500); NaOH: 204 (43000), 253 (5400), 346 (21000); HCl regenerated the original spectrum; ^{1}H nmr spectrum: δ 0.91 (m, 3H), 1.37 (m, 4H), 1.72 (m, 2H), 2.13 (s, 3H), 2.21 (s, 3H), 2.89 (t, 7 Hz, 2H), 5.32 (br. s, 1H), 7.39 (s, 1H), 13.04 (s, 1H); ^{13}C nmr spectrum: δ 7.5 (q), 13.9 (q), 15.6 (q), 22.5 (t), 24.8 (t), 31.6 (t), 37.9 (t), 110.4 (s), 112.9 (s), 114.6 (s), 129.2 (d), 158.8 (s), 161.4 (s), 205.5 (s); hreims: 236.1415 (calcd for $C_{14}H_{20}O_{3}$: 236.1413, 30%), 193 (20), 180 (29), 165 (100); cims: 237 (M+1, 100%); tlc: 0.49-0.51 (2% EtOH/$CHCl_3$), 0.68 (6%).

Octahydrotrichodimerol (**12**).

mp 147-9°C (EtOAc/Skellysolve B), 124-5°C (Bz/Et_2O); $[\alpha]_D$ +184°(*c* 0.70, MeOH); ftir υ_{max} (cm^{-1}): 3420, 2960, 2940, 2910, 1580, 1265, 1129, and 1130; uv (MeOH) λ_{max} nm (ε): 340 (390), 294 (14100); CD (MeOH) $\lambda_{extremum}$ nm ($\Delta\varepsilon$): 311 (+28), 281 (-4.9); A = +32.9; ORD (MeOH) $\lambda_{extremum}$ nm ([ϕ]): 323 (+64000), 296 (-65000); ^{1}H nmr spectrum: see Table 1; ^{13}C nmr spectrum: see Table 2; hreims: 504.2727 (calcd for $C_{28}H_{40}O_{8}$: 504.2724, 26%), 253 (65), 252 (33), 237 (17), 236 (35), 235 (25), 193 (53), 181 (76), 165 (52), 154 (73), 99 (100), 83 (19), 71 (53); cims: 502 (12%), 501 (32%), 252 (100%), 251 (73%), 233 (19%); tlc: 0.15-0.17 (2% EtOH/$CHCl_3$), 0.43-0.46 (6%).

Monoacetyloctahydrotrichodimerol (**13**).

ftir υ_{max} (cm^{-1}): 3400, 2960, 2935, 2895, 2860, 1728, 1605, 1588, 1380, 1262, 1138, 1110, 1041, 948; uv (MeOH) λ_{max} nm (log ε): 295 (4.08); NaOH: 203 (4.53), 307 (4.08); HCl regenerated the original spectrum; ^{1}H nmr spectrum: see Table 1; ^{13}C nmr spectrum: see Table 2; hreims: 546.2834 (calcd for $C_{30}H_{42}O_9$: 546.2830, 90%), 528 (19), 486 (37), 468 (14), 261 (27), 253 (42), 252 (12), 237 (22), 236 (81), 235 (52), 223 (19), 218 (21), 207 (22), 180 (33), 165 (60), 154 (21), 149 (40), 99 (100), 71 (80); cims: 564 (M+18, 29%), 547 (M+1, 14%), 504 (66), 295 (100); tlc: 0.56 (EtOH/$CHCl_3$ 1%), 0.62-0.78 (2%), 0.87-0.92 (6%).

Monoacetyloctahydrotrichodimerol (**14**).

ftir υ_{max} (cm^{-1}): 3440, 2957, 2932, 2872, 2860, 1752, 1600, 1466, 1462, 1374, 1243, 1076. uv (MeOH) λ_{max} nm (log ε): 292 (4.20); NaOH: 204 (4.69), 308 (4.20); HCl regenerated the original spectrum; ^{1}H nmr spectrum: see Table 1; ^{13}C nmr spectrum: see Table 2; hreims: 546.2822 (calcd for $C_{30}H_{42}O_9$: 546.2830, 7%), 486 (39), 443 (27), 415 (11), 291 (18), 263 (31), 251 (17), 236 (100), 218 (25), 209 (17), 192 (26), 180 (39), 167 (68), 165 (61), 99 (66), 71 (61); cims: 564 (M+18, 100%), 547 (M+1, 6%); tlc: 0.65 (EtOH/$CHCl_3$ 4%).

Diacetyloctahydrotrichodimerol (**15**).

ftir υ_{max} (cm^{-1}): 2957, 2953, 2885, 1753, 1599, 1580, 1466, 1252, 1231, 1197, 1078, 1064; uv (MeOH) λ_{max} nm (log ε): 290 (4.30); NaOH: 204 (4.74), 299 (4.34); HCl regenerated the original spectrum; ^{1}H nmr spectrum: see Table 1; ^{13}C nmr spectrum: see Table 2; hreims: 588.2931 (calcd for $C_{32}H_{44}O_{10}$: 588.2935, 93%), 447 (26), 370 (34), 317 (37), 279 (20), 278 (32), 272 (30), 263 (21), 251 (48), 237 (41), 236 (95), 235 (43), 233 (20), 223 (24), 218 (27), 192 (31), 180 (42), 167 (47), 165 (86), 99 (100); cims: 606 (M+18, 100), 589 (M+1, 22%); tlc: 0.46-0.63 ($EtOH/CHCl_3$ 2%), 0.83-0.87 (6%).

Triacetyloctahydrotrichodimerol (**16**).

ftir υ_{max} (cm^{-1}): 2956, 2932, 2880, 2865, 1752, 1699, 1612, 1465, 1373, 1253, 1232, 1193, 1176, 1066; uv (MeOH) λ_{max} nm (log ε): 224 (3.90), 229 (3.90), 255 (4.08), 292 (4.00); NaOH: 205 (4.89), 248 (4.00), 304 (4.26); HCl regenerated the original spectrum; ^1H nmr spectrum: see Table 1; ^{13}C nmr spectrum: see Table 2; hreims: 630.3069 (calcd for $C_{34}H_{46}O_{11}$: 630.3045, 5%), 604 (36), 588 (8), 468 (18), 401 (26), 370 (34), 359 (31), 317 (22), 252 (9), 251 (27), 236 (46), 235 (43), 233 (24), 218 (35), 180 (25), 165 (63), 99 (100), 83 (21), 71 (87); cims: 648 (M+18, 45%), 631 (M+1, 22%), 588 (M -42, 100%); tlc: 0.28-0.32 ($EtOH/CHCl_3$ 2%), 0.73 (6%).

Octahydrotrichodermolide (**25**).

$[\alpha]_D$ +119°(*c* 0.55, $CHCl_3$); ftir υ_{max} (cm^{-1}): 2956, 2923, 2872, 2850, 1782, 1715, 1688, 1618, 1463, 1379, 1304, 1184, 1052, 920; uv (MeOH) λ_{max} nm (ε): 219 (5600), 272 (5700), 325 (1200), 329 (1200); CD (MeOH) $\lambda_{extremum}$ nm (Δε): 270 (+13), 222 (-15); ORD (MeOH) $\lambda_{extremum}$ nm ([ϕ]): 288 (+24000), 242 (-58000); ^1H nmr spectrum: see Table 5; ^{13}C nmr spectrum: see Table 6; hreims: 404.2552 (calcd for

$C_{24}H_{36}O_5$: 404.2564, 0.8%), 262 (33), 163 (19), 99 (100), 85 (20), 71 (79); cims: 405 (M+1, 100%).

β-Decahydrotrichodermolide (**27a**).

ftir υ_{max} (cm^{-1}): 3450, 2956, 2929, 2872, 2858, 1770, 1714, 1457, 1379, 1049; uv (MeOH): no absorptions detected; ^{1}H nmr spectrum: see Table 5; hreims: 406.2737 (calcd for $C_{24}H_{38}O_5$: 406.2720, 0.8%), 361 (29), 248 (19), 245 (22), 149 (54), 147 (26), 133 (20), 121 (38), 99 (100), 71 (66); cims: 424 (M+18, 100%), 407 (M+1, 12%).

α-Decahydrotrichodermolide (**28a**).

ftir υ_{max} (cm^{-1}): 3460, 2956, 2932, 2872, 2860, 1773, 1756, 1713, 1379, 1043, 925; ^{1}H nmr spectrum: see Table 5; ^{13}C nmr spectrum: see Table 6; hreims: 406.2730 (calcd for $C_{24}H_{38}O_5$: 406.2720, 0.8%), 150 (19), 149 (22), 147 (37), 133 (28), 121 (50), 99 (100), 71 (70).

O-Acetyl-α-decahydrotrichodermolide (**27b**):

ftir υ_{max} (cm^{-1}): 2956, 2928, 2872, 2857, 1780, 1748, 1715, 1457, 1417, 1380, 1371, 1258, 1022, 931, 804; uv (MeOH): no uv absorptions detected; ^{1}H nmr spectrum: see Table 5; ^{13}C nmr spectrum: see Table 6; hreims: 448.2831 (calcd for $C_{26}H_{40}O_6$: 448.2826, 0.1%), 404 (-CO_2, 0.2), 246 (41), 147 (47), 135 (6), 133 (42), 121 (16), 99 (100), 71 (72).

O-Acetyl-β-decahydrotrichodermolide (**28b**).

ftir υ_{max} (cm^{-1}): 2956, 2927, 2873, 2858, 1772, 1742, 1716, 1374, 1231, 1093, 1056, 1028; uv (MeOH): no absorptions detected; ^{1}H nmr spectrum: see Table 5; hreims: 448.2817 (calcd for $C_{26}H_{40}O_6$: 448.2826, 0.1%), 403 (-CO_2, 8%), 248 (30), 245 (42), 133 (44), 121 (22), 99(100), 71 (71); cims: 466 (M+18, 100%), 449 (M+1, 3%).

Anhydrodecahydrotrichodermolide (**26**).

ftir υ_{max} (cm^{-1}): 2955, 2930, 2872, 2859, 1779, 1715, 1687, 1381, 1180, 1070; uv (MeOH) λ_{max} nm (ε): 202 (6000), 232 (2000); No change upon addition of NaOH or HCl; ^{1}H nmr spectrum: see Table 5; ^{13}C nmr spectrum: see Table 6; hreims: 388.2611 (calcd for $C_{24}H_{36}O_4$: 388.2615, 0.6%), 277 ($C_{18}H_{29}O_2$, 100%), 245 (21), 99 (55); cims: 406 (M+18 100%), 390 (M+2 36%).

Aromatic compound **30**.

ftir υ_{max} (cm^{-1}): 2958, 2928, 2871, 2858, 1704, 1450, 1418; uv (MeOH) λ_{max} nm (ε): 205 (13000), 210 (15000), 269 (400); no change upon addition of NaOH or HCl; ^{1}H nmr spectrum: δ 0.87 (t, 7 Hz, 6H), 1.26 (m, 8H), 1.56 (m, 4H), 2.04 (s, 3H), 2.20 (s, 6H), 2.40 (t, 7 Hz, 4H), 3.76 (s, 4H), 6.92 (s, 1H); ^{13}C nmr spectrum: δ 13.9, 16.7, 20.6, 22.4, 23.6, 31.4, 42.0, 44.9, 130.2, 130.4, 135.4, 135.8, 208.6; hreims: 344.2714 (calcd for $C_{23}H_{36}O_2$: 344.2715, 5%), 326 (21), 245 ($C_{17}H_{25}O$, 100%), 227 (24), 147 (23), 99 (44); cims: 362 (M+18, 100%), 345 (M+1, 1%), 245 (7); nOe: [H-1] (2.04 ppm): H-5 (3.76 ppm) (4.3%), Me-10 (2.20 ppm) (-3.2%); [Me-10] (2.20 ppm): H-5 (3.76 ppm) (1.5%), H-1 (6.92 ppm) (3.6%); [H-5] (3.76 ppm): Me-10 (2.20 ppm) (5%), H-6 (2.40 ppm) (4%), Me-11 (2.04 ppm) (7.3%); [H-1] (6.92 ppm): Me-10 (2.20 ppm) (13.5%).

Tetrahydrosorbiquinol (**43**).

$[\alpha]_D$ +86°(c 0.23, MeOH); ftir υ_{max} (cm^{-1}): 3450, 2960, 2870, 2850, 1730, 1625, 1490, 1380, 1292, 1165, 1145; uv (MeOH): see Table 11; ^{1}H nmr spectrum: see Table 8; ^{13}C nmr spectrum: see Table 9; CD (MeOH) $\lambda_{extremum}$ nm ($\Delta\varepsilon$): 318 (+37), 284 (-41); A = +78; hreims: 484.2460 (calcd for $C_{28}H_{36}O_7$: 484.2462, 3.3%), 252 (2), 232 (25), 165 (100), 99 (10), 95 (3); cims: 502 (M+18, 3%), 485 (M+1, 41%), 253 (32), 233 (100), 106 (82); tlc: 0.26-0.27 (2% EtOH/$CHCl_3$), 0.49-0.55 (6%).

Hexahydrosorbiquinol (**44**).

$[\alpha]_D$ +117°(c 0.23, MeOH); ftir υ_{max} (cm^{-1}): 3480, 2955, 2865, 1732, 1627, 1482, 1450, 1382, 1188, 757; uv (MeOH): see Table 11; ^{1}H nmr spectrum: see Table 8; ^{13}C nmr spectrum: see Table 9; CD (MeOH) $\lambda_{extremum}$ nm ($\Delta\varepsilon$): 316 (+29), 285 (-34); A = +63; hreims: 486.2616 (calcd for $C_{28}H_{38}O_7$: 486.2618, 17%), 253 (1.4), 235 (28), 191 (28), 165 (100), 99 (5); cims: 504 (M+18, 34), 487 (M+1, 100), 253 (78), 233 (79), 165 (54); tlc: 0.24-0.27 (2% EtOH/$CHCl_3$), 0.53-0.60 (6%).

O-Acetyl-octahydrosorbicillin (**73**).

ftir υ_{max} (cm^{-1}): 2956, 2930, 2871, 1764, 1740 sh, 1637, 1369, 1204, 1107; uv (MeOH): λ_{max} nm (ε): 216 (20000), 256 (12000), 333 (3400); base: 210 (25000), 255 sh, 347 (5000); ^{1}H nmr spectrum: δ 0.92 (m, 3H), 1.41 (m, 4H), 1.78 (m, 2H), 2.07 (s, 3H), 2.13 (s, 3H), 2.37 (s, 3H), 2.95 (t, 7 Hz, 2H), 7.49 (s, 1H), 12.72 (s, 1H) ppm. ^{13}C nmr spectrum: δ 116.8, 119.8, 120.5, 128.5, 153.6, 160.5, 205.6 ppm; hreims: 278.1515 (calcd for

$C_{16}H_{22}O_4$: 278.1512, 32%), 236 (33%), 218 (30%), 193 (24%), 180 (49%), 165 (100%).

Incorporation Experiments.

T. longibrachiatum strain UAMH 4159 was chosen for the feeding experiments since it produces high yields of trichodermolide and trichodimerol. The inocula and the cultures were prepared as described previously. Still cultures containing lactose as the major carbon source were grown for three weeks.

A stock solution containing sodium [1-^{13}C]acetate (90% ^{13}C, 2.7 g) in water (10 mL) was prepared and adjusted to pH 5 (conc. HCl). The stock solution (1.2 mL/L) was administered to the fungal cultures (2 L) on the 10th, 12th, 14th, and 16th day of growth by gentle injection of the solution at 4 points slightly under the mycelial mat. The fungus was harvested on the 22th day of growth. Extraction of dry mycelium (11.2 g) in the usual manner gave the mycelial extract (0.5 g) while extraction of the broth gave the broth extract (0.84 g).

Trichodimerol, sorbicillin, bislongiquinolide, and trichodermolide were isolated from the mycelial extract in the manner described previously. Due to the small amount of sample available sorbiquinol was isolated as tetrahydrosorbiquinol by catalytic hydrogenation of the fractions containing sorbiquinol. Bisvertinol was obtained by chromatography of the broth.

A stock solution of sodium [1,2-$^{13}C_2$]acetate (91% ^{13}C, 0.86 g)in water (10 mL) was prepared and adjusted to pH 4.5 (conc. HCl). The stock solution (2.5 mL/L) was administered to the fungal still cultures (1 L) on the 10th, 13th, 15th, and 18th day of growth by gentle injection of the solution at 4 points slightly under the mycelial mat. The fungus was harvested on the 26th day of growth. Extraction of the dry mycelium (4.8 g) as previously described gave the mycelial extract (0.26 g).

Only trichodimerol and sorbicillin were isolated from the mycelium extract.

NMR Techniques.

Natural abundance INADSYM.

Octahydrotrichodimerol (600 mg) was dissolved in deuterated benzene (1.5 mL). A ten millimeter broad-band nmr probe (90.6 MHz) was used. Sixty four FIDs of 2K points and 640 scans each were obtained with the program INADSYM* of Bruker. The delays were optimized for a carbon-carbon coupling constant of 59 Hz. A relaxation delay of 2 seconds was used between scans. This required a total spectrometer time of 40 hours. The spectral window used was of 4545 Hz, corresponding to 50 ppm at 90.56 MHz. A zero filling to 1024w was done in F1 and the spectra was Fourier transformed with a gaussian multiplication in F_1 (LB_1=-100, GB_1=0.2), and a sinusoidal multiplication in F_2 followed by symmetrization.

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* The INADSYM sequence gives tha same information as the original INADEQUATE-2D sequence, with the advantage that the resulting spectrum can be symmetrized and has a cosy-like appearance.

[1,2-$^{13}C_2$]Acetate incorporated trichodimerol 2D-INADEQUATE.

[1,2-$^{13}C_2$]acetate incorporated trichodimerol (40 mg) and chromium (III) acetylacetonate (5 mg), as a relaxation aid, were used in an overnight 2D-INADEQUATE experiment. The double quantum delay was maximized for a coupling of 33 Hz. The spectral window used was of 19230 Hz (191 ppm at 100.6 MHz). One hundred and fifteen 4K experiments were obtained and F_1 zero filled to 256w, followed by sinusoidal multiplication in F_1 and F_2.

Selective INEPT experiments.

The following pulse sequence was used for the selective INEPT experiments:

^{1}H:	D1	90	D2	180	D2	90	D3	180	D3	BB
^{13}C:				180		90		180		FID

The delay times D1 (^{1}H relaxation delay), D2, and D3 were 3.00, 0.030, and 0.025 seconds, respectively. The first 90° pulse on hydrogen was selective (45L, 90° = 18 ms), while the following pulses on hydrogen were non selective. When only selective pulses were used on hydrogen, some signals appeared out of phase.

[^{1}H]-^{1}H NOe difference experiments.

Difference spectra were obtained by subtraction of the FID (16K) corresponding to the signal irradiated and a reference spectrum obtained in the same experiment. The measured nOe is the area of the enhanced signal divided by the area of the saturated signal in the difference spectra. Typically 16 scans for every irradiation were obtained with 90° pulses, a saturation time of 4.5-6.0 seconds between pulses, and cycling through the list of irradiation points. These cycles were successively repeated at least four times and the FIDs added. The decoupler power used was 53L (Bruker scale) when methyl singlets were irradiated, and 40-45L when multiplets were irradiated. At least 2 dummy scans were used at the start of every acquisition.

$[^1H]$-^{13}C NOe difference experiment.

The same procedure used for $[^1H]$-^1H nOe experiments with a saturation time of 10 seconds was used. Four dummy and 200 scans were obtained for every irradiation and the frequency list repeated six times. The decoupler power used was 45L.

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